Quantitative Microbial Risk Assessment on the risk of listeriosis posed by locally manufactured cheese products

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Declaration

I declare that this thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgment is made in the text of the thesis.

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Abstract

This thesis presents a process-specific strategy for the direct interpretation of the cheesemaking process in terms of microbial growth, utilising elements of risk assessment and predictive microbiology. Locally manufactured fresh cheeses (Ricotta and Mascarpone) and a surface-ripened cheese (Brie) are identified as short shelf life products, susceptible to microbial contamination. A hazard analysis is used to determine that *Listeria monocytogenes* poses the main danger from the consumption of these cheeses. Several previously published predictive models for describing the behaviour of *L. monocytogenes* are evaluated by two methods: - comparison of predictions with observed growth through a series of challenge tests, and comparison with published scientific data. The results of the evaluation process demonstrate that the model of Murphy *et al.* (1996)[1], a model specifically designed by the authors for use in dairy products, is the most suitable for predictions of *L. monocytogenes* growth in these cheeses.

For each cheese product, the manufacturing process is defined in terms of the primary parameters controlling growth of *L. monocytogenes* (temperature, pH and salt concentration), and a microbiological profile of the process used to highlight potential contamination sources. A stochastic modelling approach, utilising commercial @RISK simulation software, is used to account for process variability and a distribution is defined for each parameter. A simplified modelling approach is initially used to generate a series of outcomes for each process step, rather than a single point estimation of growth. Correlations are established which quantify the degree to which each parameter influences the growth of *L. monocytogenes*, thus allowing the objective determination of high risk factors and highlighting critical areas where control must be exerted to assure food safety, an ideal implicit in the implementation of HACCP-based food safety systems.

Potential growth of *L. monocytogenes* is estimated from post-heat treatment of the milk through to the end of the shelf life of the cheese. Results from Brie manufacture demonstrate that pH development during the initial production phase is crucial in limiting potential *L. monocytogenes* growth, until brining of the cheese takes place, when the concentration of salt introduced into the product becomes the most significant factor. The inhibition due to the increased salt concentration remains

significant through the shelf life of the product. Storage temperature becomes the most significant limiting factor as the cheese matures and the pH rises after wrapping. The Ricotta manufacturing process contains no significant limiting factors and growth is limited only by the rate of cooling once the curd is scooped. The development of a lowered pH in Mascarpone manufacture has a significant effect on *L. monocytogenes* growth, as does the cooling rate.

A secondary, more rigorous modelling process is also presented, incorporating less well defined parameters such as rates of contamination, lag phase duration and exposure assessment to present a full risk assessment of the number of listeriosis cases that may result from the consumption of these cheeses. The results demonstrate that all three cheeses provide favourable environments for the growth of *L. monocytogenes*, and that control measures such as good manufacturing practices must be in place to ensure that contamination with this organism is a very rare event. The outcomes presented demonstrate that should a contamination event occur *L. monocytogenes* has the potential to grow during the manufacturing process and storage of the product leading to levels that may cause illness in susceptible consumers by the end of shelf life.

1. Murphy, P. M., Rea, M. C. & Harrington, D. (1996). Development of a predictive model for growth of *Listeria monocytogenes* in a skim milk medium and validation studies in a range of dairy products. *J. Appl. Bacteriol.* **80**: 557-564.

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Abbreviations used in this thesis

Acc	Accuracy
ADASC	Australian Dairy Authorities Standards' Committee
ADC	Australian Dairy Corporation
ANZFA	Australian and New Zealand Food Authority
Atm	Atmosphere
a_w	Water activity
BPA	Baird-Parker Agar
CCP	Critical Control Point
cfu	Colony forming units
CPS	Coagulase positive *Staphylococcus aureus*
EC	European Community
EIEC	Enteroinvasive *E. coli*
EMB	Eosin Methylene Blue agar
ETEC	Enterotoxigenic *E. coli*
EPEC	Enteropathogenic *E. coli*
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration (US)
FMM	Food Micromodel
GMP	Good manufacturing practice
GT	Generation Time
HACCP	Hazard Analysis Critical Control Point
HTST	High Temperature Short Time (pasteurisation)
ICMSF	International Commission on Microbiological Specifications for Foods
IDF	International Dairy Federation
IFST	Institute of Food Science and Technology
LGR	Lag phase : Generation time ratio
LH	Latin Hypercube (stochastic sampling)
Ln	Natural Logarithm
LPD	Lag phase duration
LSA	Listeria Selective Agar (Oxford formulation)
MAFF	Ministry of Agricultural Food and Fisheries (UK)
MC	Monte Carlo (stochastic sampling)
MIC	Minimum Inhibitory Concentration

MPD	Maximum Population Density
NACMCF	National Advisory Committee on Microbiological Criteria for Foods
NASA	National Aeronautical and Space Administration
NFPA	National Food Processors Association
NHMRC	National Health and Medical Research Council
NNDSS	National Notifiable Diseases Surveillance System
NSWDC	New South Wales Dairy Corporation
PCA	Plate Count Agar
PHLS	Public Health Laboratory Service (UK)
PMP	Pathogen Modeling Program
PRM	Process Risk Model
QMRA	Quantitative Microbial Risk Assessment
RTE	Ready-to-eat
SD	Standard Deviation
SPC	Standard Plate Count
TDIA	Tasmanian Dairy Industry Authority
TDL	Temperature data logger
TGI	Temperature Gradient Incubator
TSB	Tryptone Soya Broth
TSB-YE	Tryptone Soya Broth - Yeast Extract
UHT	Ultra High Temperature
USDA	United States Department of Agriculture
VDIA	Victorian Dairy Industry Authority
VP	Vacuum packaged

Publications

Refereed Papers

Miles, D. W. & Ross, T. (1999). Identifying and quantifying risks in the food production chain. *Food Aust* **51**:298-303.

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1. INTRODUCTION

1.1 Specialty cheese industry in Tasmania

Specialty cheese within the Australian dairy industry is defined in a general manner as any non-cheddar cheese. Recent years have seen an increase in the number of cheese factories, predominantly through the establishment of small or "farmhouse" cheese companies, a trend also witnessed overseas (Coveney *et al.,* 1994). This has led to greater diversity in the range of cheeses produced and is reflected in the changed profile of cheese consumption within Australia. In 1965 non-cheddar cheeses accounted for only 7% of total cheese consumed, but in 1996-97 these varieties comprised 40% of the market (Willman, 1998). The state of Tasmania accounts for approximately 9% of Australia's total cheese production (Willman, 1998), more than larger states such as Queensland, New South Wales and Western Australia. The production of specialty cheeses has become a rapid growth industry because of the premium prices these high quality products can command, becoming very profitable for cheesemakers to produce.

In past years, with hard cheddar cheeses as the predominant product, the low pH, low moisture and longer ripening times associated with these cheeses tended to serve as safety features, controlling the growth of any pathogenic microorganisms which may have contaminated the product. With the recent trend towards increased production of surface-ripened and fresh cheese varieties, products which are characterised by high pH and moisture values, and little or no ripening periods, the conditions exist where pathogenic bacteria will potentially grow during the shelf life of the product. These types of cheeses are considered to be high risk for some consumers (Johnson, 1990a). Since most specialty cheeses are consumed as ready-to-eat products, and as no further heat treatment is given to the product by the consumer, any cheese which contains an infective dose of pathogens will cause illness in susceptible consumers. It was on this basis the cheese products used in this study were selected.

The importance of the dairy industry to the economy of the small state of Tasmania cannot be overstated. Production of dairy products is valued at over $270 million per annum, with about 2000 people directly involved in milk production, and a further 1600 in the processing and manufacture of dairy products (TDIA, 1997). In 1998, 18 licenses were issued by the regulatory agency, the Tasmanian Dairy Industry Authority (TDIA) for factories to produce cheese within the state, including ten "farm" cheesemakers. The majority of cheese producers in the state are classified as

small (<10,000 L milk/day). However, the state is home to two of Australia's best known soft-ripened cheese manufacturers, Lactos Pty. Ltd. and King Island Dairies, and thus there is a very important reputation to uphold. Despite the relatively small production volumes of most Tasmanian factories, the importance of quality assurance and food safety plans within these premises is not diminished. It is generally acknowledged that a problem, even if isolated to just one small business, can have a detrimental effect on the entire industry.

To maintain Tasmania's reputation for high quality specialty cheese products, a proactive approach has been taken by the TDIA. This required the development of food safety plans based on Hazard Analysis Critical Control Point (HACCP) principles for all cheese factories, to ensure the continued safe production of speciality cheeses. The need for improved quality assurance has been illustrated on several occasions in the past few years. In mid 1995 one company recalled 12 tonnes of shredded cheese contaminated with *Listeria monocytogenes* (Figure 1.1), while another cheese factory had its licence revoked in 1997 by the TDIA and a quantity of cheese confiscated for serious breaches of hygiene.

The TDIA has been active in assisting smaller businesses in establishing HACCP plans, and ensuring good manufacturing practices are maintained, through routine inspections carried out by the Quality Assurance officer. However, the limitations of the inspection approach are recognised, therefore the preventative approach offered through the implementation of HACCP systems provides a more thorough method for maintaining food safety. To date, HACCP-based food safety systems have been mainly applied in a qualitative fashion, resulting in subjective determinations of what constitutes a hazard, and the Critical Control Points (CCPs) and Critical Limits needed to control the hazard (Christian, 1994). Quantitative Risk Assessment has been promulgated as a method to highlight objectively the main determinants of risk to a food operation and the consumers of that food. But to date the usefulness of this strategy has been limited by the amount of process data available. This project was formulated with the aim of empowering the application of HACCP through the use of predictive microbiology and risk assessment. The inclusion of quantitative process data in the system is essential, allowing assessment of the contribution of each process stage to the overall risk the product poses to the consumer. An assessment of this type assists in determining the most effective and appropriate risk management factors.

Figure 1.1 – Local newspaper publicity concerning cheese recall in 1995

2/8/95

Shredded cheese recall because of infection fear

By JANE LOVIBOND

UP to 12 tonnes of Tasmanian shredded cheese is being cleared from supermarket shelves and household refrigerators because of the threat of contamination by a life-threatening bacteria.

United Milk Tasmania Ltd is recalling packs of the cheese distributed in Queensland and Tasmania last month after routine tests found *Listeria monocytogenes* in samples.

State Director of Public Health Mark Jacobs has issued a warning to pregnant women, the elderly and people with weak immune systems that they are particularly vulnerable to the bacteria.

He said it could bring on miscarriages and stillbirths.

Listeria causes the illness listeriosis, which in extreme form can result in meningitis and septicaemia.

Milder symptoms include fever, headaches, aches and pains, vomiting and diarrhoea.

Mr Bryan Ward: "recall a precautionary measure".

UMT general manager Bryan Ward said the bacteria had been found in two packs of test samples but it was decided to recall all shredded cheese as a precautionary measure rather than try to isolate the suspect batches.

Further tests had detected no more cases of the bacteria. However, as a double check the samples would be sent to Melbourne for testing in an independent laboratory.

Mr Ward said the recall applied to the UMT, Table Cape, Homebrand and Black and Gold labels.

Refunds would be given for all products returned to their place of purchase.

Dr Jacobs said the bacteria was common in the environment and investigations into the source of the UMT contamination were continuing.

"Listeria is destroyed by cooking but can easily be consumed when cooked and raw foods are mixed," he said.

"It is an issue in the home and consumers should heed use-by dates on food and not use the same implements when preparing raw and cooked food."

Purity Supermarkets managing director Michael Kent said packs of the suspect cheese were removed from 27 Purity and Roelf Vos stores on Monday.

Those in question were UMT-produced 500-gram packs of shredded tasty cheese, light tasty cheese and shredded Homebrand cheese.

Contaminated shredded cheese recalled by UMT

NORTH-WEST cheese producer United Milk Tasmania Ltd has recalled packs of shredded cheese after tests revealed contamination by bacteria which can cause food poisoning.

The bacteria was detected in two samples of the cheese which carries UMT, Table Cape, Homebrand and Black and Gold brands.

Packages of the suspect product have been sold in Tasmania and Queensland.

UMT general manager Bryan Ward said no illness had been reported but rather than isolate the suspect production batches all shredded cheese had been recalled. He said tests showed the presence of the bacteria listeria monocytogenes.

1.2 Cheese as a carrier of foodborne disease

Cheese has been the vehicle of several large foodborne disease outbreaks over the last 30 years, with these outbreaks being responsible for over 8000 cases of food poisoning and almost 100 deaths (Table 1.1). Some of the responsible organisms include *Brucella*, *Listeria monocytogenes*, *Shigella*, *Clostridium botulinum*, *Salmonella*, *Staphylococcus aureus*, and *Escherichia coli* (Altekruse, 1998). Despite the number of outbreaks, dairy products in general are still regarded as a very safe class of food. According to Johnson *et al.* (1990a), dairy products account for only 1-3% of reported outbreaks of foodborne disease in the United Sates, and the general consensus is they are among the safest foods consumed (Boor, 1997). However, pathogenic microorganisms are frequently found in raw milk supplies, and the temperatures used in cheese manufacture during the normal ripening of milk or cooking of the curd are not sufficiently high to destroy these organisms. The Australian dairy industry relies on heat treatment of the cheese milk, primarily High Temperature Short Time (HTST) pasteurisation (minimum 72.2°C for 15 seconds), as the main method to prevent pathogens in the final product, with the use of raw milk for cheesemaking currently prohibited (ANZFA, 1999). The majority of outbreaks resulting from cheese consumption around the world have been due to the use of unpasteurised milk, or subjected to faulty pasteurisation (Johnson *et al.*, 1990a). However, post-pasteurisation contamination can also pose a major problem where good manufacturing practices are not employed (Zottola & Smith, 1991).

1.2.1 Cheese manufacture

During the manufacture of cheese, the protein in milk is coagulated, resulting in a solid phase (curd) and liquid phase (whey). Expulsion and drainage of the whey then concentrates the curd. The manner in which these two steps are carried out affect the final moisture content of the cheese (Davis, 1976). Slight variations in pH and moisture, as well as ripening methods lead to the large number of cheese varieties available today. The moisture content is one factor influencing growth of microorganisms in the final product. Another very significant contribution to the control of pathogens in the making of the cheese arises from the lactic acid bacteria or starter cultures. These organisms grow during cheesemaking, converting lactose to lactic acid. The generation of acid by these bacteria reduces the pH, aids in whey expulsion from the curd, affects cheese flavour and inhibits the growth of pathogenic bacteria. This is one of the control methods available to the cheesemaker to prevent disease outbreaks caused by pathogens in cheese.

Table 1.1 – Food poisoning outbreaks from consumption of cheese 1970 - 1998

Year	Country	Cheese	Pathogen	Cases	Deaths
1971	USA	Camembert	EPEC	387	
1973	USA	Queso fresco	*Brucella melitensis*	3	0
1974	France, Switzerland	Soft cheese	*C. botulinum*	77	
1975	USA	Queso blanco	unknown	17	
1976	USA	Cheddar	*S.* Heidelberg	339	0
1977	Canada	Swiss	*S aureus*	12	
1980	Canada	Cheese curds	*S aureus*	62	
1981	USA	Mozzarella	*S* Typhimurium	321	2
1981	USA	Direct set cheese	*S aureus*	16	0
1981	Italy	Mozzarella	*S* Typhimurium	100	0
1982	Scandinavia	Brie	*Bacillus, Shigella*	>100	
1982	Canada	Cheddar	*Salmonella*		
1983	France	Brie	EPEC	>350	
1983	Netherlands, Denmark,, USA	Brie	ETEC	>3000	0
1983	USA	Queso fresco	*S. equi, S zooepidemicus*	16	3
1983	UK	Cheddar	*S. aureus*	2	
1983	Finland	"Farmhouse"	*Salmonella*	35	
1983-7	Switzerland	Vacherin	*L. monocytogenes*	122	34
1983	USA	Brie/Camembert	ETEC	170	
1983	USA	Queso fresco	*Brucella melitensis*	31	1
1983	Greece	Homemade	*Brucella*	23	
1984	Canada	Cheddar	*S* Typhimurium	>2700	1
1984-5	Scotland	Sheep's milk cheese	*S aureus* enterotoxin	>13	0
1985	USA	Queso fresco	*L. monocytogenes*	152	52
1985	Switzerland	Vacherin	*Salmonella*	215	
1985	USA	Queso fresco	*Brucella melitensis*	9	0
1988-9	England	Stilton	*S aureus* enterotoxin	155	0
1989	England	Irish soft cheese	*S.* Dublin	42	
1989	USA	Mozzarella	*S.* Javiana, *S.* Oranienburg	164	0
1992	England	Cheese	*S* Livingston	10	0
1992	USA	Mexican goat's cheese	*Brucella melitensis*	11	0
1992-3	France	Fromage frais	VTEC	unknown	1
1993	France	Goat's milk cheese	*Salmonella paratyphi B*	273	1
1994	Scotland	Farm cheese	VTEC	>20	0
1995	France	Brie de Meaux	*L. monocytogenes*	20	4
1995	Malta	Soft cheese	*Brucella melitensis*	35	1
1995	Switzerland, France	local cheese	*S* Dublin	25	5
1996	England, Scotland	Cheddar	*S.* Gold-coast	>84	0
1996	Italy	Mascarpone	*C botulinum*	8	1
1997	England	Lancashire	*E coli* O157	2	0
1998	Canada	Cheddar	*S* Enteritidis	>700	

Adapted from (D'Aoust, 1989; Altekruse *et al*, 1998; IFST, 1996; Health Canada, 1999)

ETEC = Enterotoxigenic *E. coli* VTEC = Verotoxigenic *E coli,* EPEC = Enteropathogenic *E. coli*

1.2.2 Hard cheeses

Hard cheese varieties, such as Cheddar, have been responsible for foodborne outbreaks in the past (as shown in Table 1.1), but with the recent improvement in starter culture technology, and therefore appropriate pH development in the cheese, these products are now considered to be low risk to the consumer (Johnson *et al.*, 1990a). Organism which are poor competitors, such as *Staphylococcus aureus*, are inhibited by the number of starter bacteria, while the long ripening times involved with the production of hard cheeses results in any contaminating pathogens dying off during this period. In cases where hard cheeses have caused food poisoning outbreaks, it has mainly been due to pathogens such as *Salmonella*, which can have a very low infective dose (D'Aoust *et al*, 1985). Therefore, growth of these pathogens does not need to occur in the product in order to cause disease.

1.2.3 Surface-ripened cheese

Surface-ripened cheeses, including well-known varieties such as Brie and Camembert, are ripened from the outside inwards due to the addition of mould cultures (*Penicillium camemberti* or *Penicillium candidum*). These are added to the outside of the shaped curd and grow during the maturation phase allowing enzymes to penetrate into the curd. The pH on the surface of the cheese is around neutral at packaging. However, the pH within the curd at packaging is still acidic, rising as the mould influences the ripening of the cheese. Due to the conditions present in soft cheeses, a number of pathogens are able to grow in the later stages of ripening (Little & Knøchel, 1994; Ryser & Marth, 1987b). As shown in Table 1.1, a large number of cases of foodborne illness have been associated with consumption of Brie, Camembert and similar varieties of cheese. These products usually have a relatively short shelf life and are considered to represent the highest risk of all cheeses. The growth of pathogens in these types of cheese is associated with the rise in pH due to proteolysis, although nutritional factors may also be involved (Ryser & Marth, 1987b). The data in Table 1.1 include cheeses made from unpasteurised milk, a common practice in many European countries. Nevertheless, it is intuitive that surface-ripened cheeses are responsible for causing many of the cheese-borne outbreaks.

1.2.4 Fresh cheese

Fresh, or unripened, cheeses are a variety in which starter cultures are not used, and therefore usually have high pH values. They are short shelf life products, often vacuum packaged to extend the shelf life. Whey cheeses are an example of fresh

cheeses, as they are made by direct heating of whey with no ripening period. There are Greek varieties of whey cheeses (Papageorgiou *et al.*, 1996); Myzithra, Anthotyros, and Manouri, as well as Italian types, of which Ricotta is most popular. Other types of fresh cheese are the cream cheeses such as Mascarpone. Most fresh cheeses usually contain very few factors in the product to retard pathogen growth and therefore product safety is mainly dependent on storage temperature.

1.2.5 Pathogens in cheese

A wide variety of pathogenic bacteria can be present in raw milk (Eyles, 1992), however Johnson *et al.* (1990b) identified *Salmonella, Listeria monocytogenes* and enteropathogenic *Escherichia coli* as the three high-risk organisms to the cheese industry. This appears to be an accurate account of the situation, given that these three organisms have caused the majority of food poisoning outbreaks arising from consumption of cheese (Table 1.1). Along with good manufacturing practice, quality assurance plans such as Hazard Analysis Critical Control Point (HACCP) systems are being implemented throughout the cheese industry to control the contamination by pathogens. HACCP will be discussed in detail in Section 1.3, with the three pathogens mentioned above considered in a hazard analysis.

1.3 HACCP - Hazard Analysis Critical Control Point

1.3.1 Background

The HACCP approach is a preventative system of food safety quality control, rather than the reactive approach utilised previously through end-product testing and factory inspection. The preventative measures associated with HACCP implementation supercede traditional methods for ensuring food safety. Factory inspections can never be conducted frequently or thoroughly enough to provide a high degree of food safety. The timing of the inspection may be such that, it fails to detect if critical processes are not being carried out correctly in the preparation of high-risk foods. Microbiological testing is also limited statistically, both by the amount of time needed (particularly for short shelf-life products) and number of samples which need to be tested to ensure complete food safety.

HACCP acknowledges safety and quality cannot be tested into a food, and therefore sets up a rational system where attention is focused on the key risk-contributing factors of the process. HACCP plans are not the solution to all process safety issues, prerequisite programs such as sanitation and good manufacturing

practice (GMP) will always play a large part in supporting HACCP. The preventative approach offered by HACCP is being seen as the best way forward, and is being adopted on a worldwide basis. It has become mandatory for all food establishments in the European Community to apply HACCP principles (European Community, 1993). In Australia, ANZFA (Australia New Zealand Food Authority) and other state-based regulatory authorities are in the process of ensuring all food processors have food safety plans in place, based on HACCP principles.

The initial development of HACCP can be traced back to the USA in the 1960's, where the Pillsbury Company researched food production for use by NASA in their space program (Bauman, 1992). For this purpose 100% assurance was needed, to ensure that the food was not contaminated with any bacterial or viral pathogens, the effect of which could result in an aborted mission. The amount of end-product testing needed for this level of assurance meant a large part of each production batch was used for testing, with only a relatively small portion left available for space flights. The original HACCP approach was 'borrowed' from field of fault scenario analysis used by engineers when designing systems with nil tolerance of failure. This led to the formulation of HACCP as a basis for a preventative system, which has since been adopted as a uniform approach to food safety within the food industry. There are seven principles associated with the full implementation of a HACCP system, presented in Table 1.2.

The HACCP system has been applied to all parts of the food production chain, including farm management (Sischo *et al.*, 1997), food processing (Peta & Kailasapathy, 1995; Kim *et al.*, 1998), retail (Reimers, 1994) and restaurant environments (Bryan, 1990). The initial focus involves the raw ingredients, identifying potential contaminants such as microbial pathogens, heavy metals, toxins, physical hazards and chemicals. The next consideration includes the type of treatments the ingredients may receive, such as pesticide applications or a pasteurisation step. Analysis of the manufacturing process is then undertaken along with a general understanding of the building, general environment and method of personnel control. This is done to ensure all points of the process which may contribute to a hazard are completely understood. A good HACCP system will also investigate procedures to prevent a hazard occurring. A final part of the investigation is examination of the conditions of storage, transportation and distribution for the product and possible abuses it may receive. An analysis to determine what consumer practices which may lead unsafe conditions may also be conducted.

Table 1.2 - HACCP principles

1. Assess hazards associated with raw materials and ingredients, processing, manufacturing, distribution, marketing, preparation and consumption of the food.
2. Determine the Critical Control Points required to control the identified hazards.
3. Establish the critical limits which must be met at each identified CCP.
4. Establish procedures to monitor critical levels.
5. Establish corrective action to be taken when there is a deviation identified by monitoring of a Critical Control Point.
6. Establish effective recordkeeping systems that document the HACCP plan.
7. Establish procedures for verification that the HACCP system is working correctly.

Adapted from ICMSF (1988)

1.3.2 HACCP Principle #1 - Hazard Analysis

The first step of any HACCP system is a hazard analysis, involving an assessment of hazards associated with all aspects of production of the particular food product of interest. A hazard, as defined by the National Advisory Committee on Microbiological Criteria for Foods, is "any biological, chemical, or physical property that may cause an unacceptable consumer health risk" (NACMCF, 1994). An extensive list of all three hazard types is presented in Rhodehamel (1992). Physical hazards are the most commonly detected, because there are numerous chances for contamination with foreign material along the process line. However, biological hazards receive more attention because of the potential for growth in the food and, therefore, to affect more people. It is beyond the scope of this thesis to consider physical or chemical hazards that may be associated with the cheeses being considered here and only microbiological hazards will be investigated. Microbiological hazards can be further divided into four types: bacterial, viral, mycological and parasitic. This thesis is concerned with bacterial hazards and the risk they pose to the consumer

1.3.2.1 Bacterial hazards

There are four situations which may lead to the emergence of a bacterial hazard:

1. Raw materials or food ingredients which can be regarded as potential sources of pathogens, food spoilage organisms, or toxic substances (e.g. pre-formed toxins).

2. Sources of contamination during production, processing or distribution.
3. Manufacturing processes lacking a controlled processing step that effectively destroys relevant microorganisms.
4. Steps during production, processing, distribution, storage, etc. which provide an opportunity for microorganisms to survive or grow.

HACCP systems are formulated with the aim of controlling biological hazards in the following manner: (1) destroy, eliminate, or reduce the hazard; (2) prevent recontamination; and (3) inhibit growth and toxin production. Many schemes have been published to aid in the identification of bacterial hazards (Rhodehamel, 1992; ICMSF, 1996; Boor, 1997; van Gerwen *et al.*, 1997) with hazardous microorganisms ranked according to their severity. Group I are classed as severe hazards; Group II are moderate hazards (although the illnesses in certain susceptible populations or complications can be severe) with the potential for extensive spread. Pathogens in Group III cause common-source outbreaks with subsequent spread usually rare (Rhodehamel, 1992).

Many food products have a unique microbiology and group of associated bacterial pathogens. Processors of specific foods (e.g. dairy) are required to consult reference materials in those areas (Zottola & Smith, 1991; Boor, 1997). To conduct an appropriate hazard analysis requires both the determination of *risk* (the probability of the potential hazard being realised) and the *severity* of an outbreak. This is important in establishing a rank order of points requiring consideration. As stated in Section 1.2.5, Johnson *et al.* (1990b) defined three organisms as being high risk to the cheese industry: *Salmonella*, enteropathogenic *E. coli* (EPEC) and *Listeria monocytogenes*. The hazard assessment conducted by those authors was based on epidemiological data, pathogen incidence in milk and characteristics of the pathogen (Johnson *et al.*, 1990b). Each of these three high-risk organisms will be considered in turn to determine which most likely constitutes the most serious hazard to the short shelf life cheese products under consideration in the current study.

1.3.3 *Salmonella*

Salmonellae are gram-negative, non-spore forming rods, usually 0.7-1.5 μm in diameter and 2-5 μm in length, belonging to the family Enterobacteriaceae. They are facultatively anaerobic and most types are motile. Salmonellae are classified by serovar (serotype) names, and although all are considered to be pathogenic, serovars differ in the pathological syndromes they produce and in their host adaptations.

Salmonellae can enter milk by faecal contamination of the udder and teats, or by contamination of the milk-handling equipment (McManus & Lanier, 1987), and can be found in the dairy environment (Cotton & White, 1992). Pasteurisation of the milk will destroy any contaminating salmonellae, but use of sub-pasteurisation temperature may be potentially hazardous (D'Aoust *et al.*, 1987). *Salmonella* outbreaks have arisen from both hard and soft cheeses, mostly due to virulent strains with low infective doses required to cause illness (D'Aoust *et al.*, 1985).

1.3.3.1 Growth limits

On foods most salmonellae do not grow at temperatures below 7°C (Jay *et al.*, 1997). Generation times at 10°C on meat have been observed to be quite long (8-26 hr), compared to spoilage organisms. The optimum temperature for growth is 35-37°C, with a maximal growth temperature of 49.5°C (ICMSF, 1996). Most salmonellae are sensitive to heat (D'Aoust *et al.*, 1987), and heat resistant strains are uncommon (Jay *et al.*, 1997). However, Mackey & Derrick (1987) reported heat resistance may be increased when the medium is heated slowly. Salmonellae can survive on foods for long periods of time at chill temperatures with small numbers persisting for significant periods, causing D'Aoust (1989) to criticise as ineffective the United States Food and Drug Administration's recommended practice of ageing raw milk cheese for 60 days at less than 4.4°C.

The pH range for growth of *Salmonella* in laboratory media is from 4.0 up to 9.5, with the optimum in the range 6.5 - 7.5 (ICMSF, 1996). In broth acidified with lactic acid the minimum pH for growth was found to be 4.4 (Chung & Goepfert, 1970). Salmonellae are destroyed or inactivated during the fermentation of high-acid products (1% lactic acid, pH value less than 4.55), although the effect is less in cheese because of the protective effect of casein. Growth of salmonellae may occur in the curd of low-acid cheese (pH value greater than 4.95). The numbers of salmonellae decline during ripening, the effect being greatest at higher temperatures. Most salmonellae are able to grow aerobically in laboratory media from an a_W of 0.999 to below 0.960 (Jay *et al.*, 1997).

1.3.3.2 Clinical symptoms

Two clinical syndromes are generally recognised in human *Salmonella* infections. Enterocolitis is the clinical manifestation of human salmonellosis caused by non-typhoid organisms. This disease has clinical symptoms which may include non-bloody watery diarrhoea, abdominal pain and nausea which appear 8-72 hr after

ingestion of the infectious agent. Other symptoms may include mild fever, vomiting and prostration, with symptoms lasting 2 to 5 days. In immunocompromised patients there may also be the serious complication of septicaemia (Jay *et al.*, 1997). Enteric fever is a severe systemic infection caused by *S.* Typhi and the closely related paratyphoid organisms in which the clinical symptoms appear 8-28 days after infection with these highly virulent and invasive strains. Spiking fever and abdominal cramps coupled with underlying bacteraemia are characteristically encountered in the first week of the disease, whereas watery diarrhoea (or constipation) with persistent abdominal pain will generally prevail in the second week of illness.

1.3.3.3 Behaviour of Salmonella *in cheese*

Salmonellae are among the most resistant of the enteric pathogens to drying, freezing, low pH and storage under dry and cold conditions. Thus, faulty pasteurisation practices may permit these organisms to persist in the milk, to grow during the initial phase of cheesemaking, and remain viable during ripening.

The number of large outbreaks from cheese are mostly attributable to the production of cheese from thermised milk (62°C for 15 seconds) or raw milk (D'Aoust, 1994). Although large numbers of people have become ill as a result of salmonellae outbreaks, there have been relatively few deaths recorded from cheeseborne outbreaks (Table 1.1). However, the risk of illness may be increased through prolonged temperature abuse of raw milk on the farm, during transportation and storage before processing. This could lead to growth to high numbers of salmonellae that may exceed the efficacy of the pasteurisation process. Infective doses from cheese have been stated to be as low as 1-10 cells for *S.* Typhimurium, and 100 cells for *S.* Heidelberg (D'Aoust *et al.*, 1985; D'Aoust, 1994). Foods of high fat content, such as cheese, can protect salmonellae against the antibacterial action of gastric secretions by encapsulating the pathogen in a lipid micelle. This can allow the organism safe passage through the stomach into the intestinal tract where it may multiply and invade underlying tissues (Jay *et al.*, 1997).

Little & Knøchel (1994) examined the growth of *S.* Dublin, *S.* Thompson and *S.* Typhimurium in Brie stored at a range of temperatures. The Brie surface had a pH of 6.8 and contained 2% NaCl (calculated $a_w = 0.989$). The strains grew at 20°C with generation times of 6.0, 4.5 and 5.9 hrs respectively. At refrigeration temperatures of 4 and 8°C growth was inhibited, but the organism did survive for extended periods of

time, and could therefore pose a hazard should the cheese be temperature abused. Papadopulou *et al.* (1993) investigated the behaviour of *S.* Enteritidis during the production of Feta cheese made from raw ewe's milk. The organism grew during the manufacturing phase of the process because of the favourable conditions present, but then gradually died during the first 20 days of ripening. The final pH of the cheese was 4.35, and the a_w was 0.935. During the manufacturing phase of cheddar cheese, any salmonellae which may be present can grow, because the pH fall produced by the action of the starter culture is insufficient to inhibit growth. However, growth ceases during salting because of the combination of lowered pH and a_w. Slow acid-production, resulting in a high cheese pH, allows the organism to survive for longer, D'Aoust *et al.* (1985) stated *S.* Typhimurium can survive for up to 8 months in Cheddar cheese at 5°C.

1.3.4 Pathogenic *E. coli*

E. coli are gram-negative, non-spore forming rods, usually 0.7-1.5 μm in diameter and 2-5 μm in length, belonging to the family Enterobacteriaceae. They are facultatively anaerobic and most types are motile. A number of *E. coli* strains can cause gastroenteritis or diarrhoeal disease in humans and other animals. These types are classified into a number of groups including verotoxigenic (VTEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC) and enterohaemorrhagic (EHEC) (Desmarchelier & Grau, 1997). Enteropathogenic *E. coli* outbreaks from consumption of French surface-ripened cheeses (Marier *et al.*, 1973; MacDonald *et al.*, 1985) were caused by a very virulent strain with a low infective dose, but no outbreaks have occurred from hard cheese varieties.

1.3.4.1 Growth limits

The minimum temperature for growth of *E. coli* is about 7 -8 °C and the maximum temperature for growth is near 49°C (Salter *et al.*, 2000). Palumbo (1995) reported verotoxin production from haemorrhagic *E. coli* strains could occur at temperatures as low as 10°C and as high as 49°C. Pathogenic strains of *E. coli* are no more heat sensitive than their non-pathogenic counterparts, a D-value of 0.78 min in cow's milk at 58°C has been reported for one strain (Desmarchelier & Grau, 1997). Although it does not grow at refrigeration temperatures lower than 7°C; *E. coli* survives well in chilled and frozen food (Desmarchelier & Grau, 1997).

The pH tolerance of *E. coli* ranges from 4.4 to 10, with the optimum in the range 6 - 7. Concern has been expressed regarding acid tolerance of pathogenic *E. coli* strains due to several outbreaks from acidic foods such as yoghurt, apple juice and soft cheese. Subsequently, much research was conducted into the behaviour of pathogenic *E. coli* strains in acidic foods (Glass *et al.*, 1992; Hathcox & Beuchat, 1995; Clavero & Beuchat, 1996; Massa *et al.*, 1997). There appears to be a large variation in acid tolerance between strains, both within pathogenic and non-pathogenic strains. Brown (1997) related the degree of intrinsic acid tolerance to the proportion of cyclopropane fatty acids in the bacterial membrane. The minimum a_W for growth of *E. coli* is about 0.95 (8% NaCl), but at lower temperatures the amount of salt required to prevent growth is lessened (Salter *et al.*, 2000).

1.3.4.2 Clinical symptoms

ETEC strains are a major cause of diarrhoea in infants and are a leading cause of traveller's diarrhoea. Illness is characterised by watery diarrhoea, low-grade fever, abdominal cramps, malaise and vomiting. It can also bring about a cholera-like illness in a more severe form. The disease caused by EIEC strains is similar to shigellosis with symptoms including chills, fever, headache, muscular pain, abdominal cramps and diarrhoea. EPEC strains are responsible for an illness with watery diarrhoea, fever, vomiting and abdominal pain. Most of the illness caused by these strains is self-limiting, but the symptoms associated with EHEC strains can be much more serious. As well as mild diarrhoea, some infections can result in haemorrhagic colitis (HC), haemolytic uraemic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP) and death (Desmarchelier & Grau, 1997).

During the early 1970's a series of outbreaks of EPEC gastroenteritis, along with ETEC and EIEC, occurred in the USA from Camembert cheese imported from France (Marier *et al.*, 1973). In this outbreak there were 107 episodes of gastroenteritis involving at least 387 persons. The outbreak was traced to *E. coli* O124:B17, and the attack-rate for exposed persons was 94%. The infective dose was reported as 10^6 - 10^8 organisms. Counts on the incriminated cheese showed it contained 10^5 -10^7 cfu/g. These were the first documented outbreaks of foodborne illness caused by *E. coli* in the United States, but a number of earlier outbreaks had been reported in other countries. The U.S. outbreaks caused considerable concern about cheese as a vehicle for transmission of EPEC diarrhoeal disease to humans. There is strong evidence EPEC is able to grow to hazardous levels in cheese if it is

present in the early stages of manufacture (Kornacki & Marth, 1982; Reitsma & Henning, 1996; Quinto & Cepeda, 1997). Also contributing to this concern, coliforms including *E. coli* are often present in various cheeses, occasionally in high number. ETEC caused an outbreak in several states of the US in 1983 (MacDonald *et al.*, 1985), where the product implicated was imported French Brie cheese, with an attack rate of 75%. The same cheese was also implicated in European outbreaks. *E. coli* O27:H20 producing ST (Shiga-like Toxin) enterotoxin was isolated from patients, but the enterotoxin could not be isolated from the cheese. Although the number of people infected during cheeseborne *E. coli* outbreaks has been quite high (attack rates over 90% were reported by Marier *et al.* (1973)), very few deaths have resulted from these outbreaks (Table 1.1.).

1.3.4.3 Behaviour of E. coli *in cheese*

Lactic fermentation of milk is inhibitory to the growth and survival of toxigenic *E. coli*, however growth of the organism generally occurs during the first 6 hours, the extent dependent upon the level of starter inoculum and the temperature of incubation. In the case of impaired starter activity, populations of *E. coli* approached 10^9/g in 24 hr and then declined shortly thereafter (Kornacki & Marth, 1982). Semisoft, surface-ripened cheeses are particularly susceptible to abnormal fermentations because of slow or non-existent acidity development during manufacture, and low salt levels in the interior of the cheese during the early stage of ripening.

Reitsma & Henning (1996) showed *E. coli* O157:H7 could survive, and even grow during the manufacture of Cheddar cheese, and was still present at the end of the 60 day curing period. Kornacki & Marth (1982) investigated the behaviour of several enteropathogenic strains of *E. coli* during the manufacture of Colby cheese. Numbers of all strains increased 1000-fold during the initial steps of cheesemaking, with generation times in the order of 20-30 minutes, achieving maximum populations of 10^6 cfu/g. During the curing phase numbers declined, except in one case where one strain persisted at levels of 10^3 cfu/g for 12 weeks of refrigerated storage. Poor quality cheese was also manufactured to test how this affected the organism. The higher than normal pH allowed levels of 10^8 cfu/g to be reached by the third day of manufacture and persist for 12 weeks.

In a survey of Spanish soft cheeses made with raw and pasteurised milk Quinto & Cepeda (1997) found three positive samples out of the 221 raw milk cheeses tested, and none from the 75 pasteurised milk cheese samples. Therefore pathogenic *E. coli* strains appear to be relatively rare in cheese. However, it appears these organisms can pass from the cow into the milk and survive the soft cheese manufacturing if made from raw milk. Quinto & Cepeda (1997) showed 17% of sampled cheeses contained faecal coliforms in excess of 10^4/g (maximum $2x10^6$/g). The occurrence of high numbers of faecal coliforms in soft and semisoft cheeses represents a potential pathogenic *E. coli* hazard. *E. coli* can grow well on the surface of Camembert (MacDonald *et al*, 1985), therefore special care should be taken to prevent surface contamination, and the temperature during the development of the mould covering should be as low as practical (not higher than 10-11°C).

1.3.5 *Listeria monocytogenes*

Listeria monocytogenes cells are gram-positive, short, regular rods with rounded ends and can occur singly, in parallel or in short chains arranged to form a V-shape. The cells are 0.4-0.5 μm in diameter and 0.5-2.0 μm in length. Gram variability may occur in isolates grown in artificial media longer than 24 hours. In older cultures, filamentous cells 6-20 μm in length may be observed. The organism is motile by means of a few peritrichous flagella when grown at 20-25°C. Cells grown in liquid media at 20-25°C have a characteristic and highly active, tumbling motility when observed under the microscope.

L. monocytogenes will grow in most bacterial culture media. Growth is enhanced in the presence of glucose, serum and blood. A variety of selective media have been suggested for the isolation of the organism from food (Swaminathan *et al.*, 1988; Cassiday *et al.*, 1989; Lachica, 1990; Tran & Hitchins, 1996; Vlaemynck & Moermans, 1996). Recently developed selective agar media rely on differential characteristics such as aesculin hydrolysis and mannitol fermentation. Colonies on many of these selective agar media develop a highly characteristic depressed button centre.

1.3.5.1 Growth limits

L. monocytogenes is able to grow at low temperatures, down to 1°C (Walker *et al.*, 1990) and this poses a serious threat to the food industry and particularly manufacturers of dairy products (Ryser & Marth, 1991). The optimum temperature is

between 30 to 37°C, while the upper limit is 45°C and the lower limit 1°C (ICMSF, 1996). It does not survive batch heating at 60°C for 30 minutes.

The pH limits of *L. monocytogenes* have been stated as being 4.6 to 9.6 (Sutherland & Porritt, 1997). Studies concerning the effect of pH on growth and/or survival of *L. monocytogenes* in various cheeses indicate the organism can grow to levels of ~10^6-10^7 cfu/g in 65 day old fully ripened Camembert cheese at pH 5.9-7.2, survive for 70 to 434 days in Cheddar cheese at pH 5.00-5.15, and >115 days in Colby cheese at pH 5.00-5.18 (Ryser & Marth, 1991). Sutherland & Porritt (1997) stated it is unlikely that growth will occur in fermented dairy products with pH values below 5.2.

L. monocytogenes grows optimally at $a_W = 0.99$, however it can also multiply at a_W levels as low as 0.92. *L. monocytogenes* can grow in laboratory media with up to 10% (w/v) NaCl ($a_W = 0.935$), however reports of growth in broth with up to 12% (w/v) NaCl ($a_W = 0.920$) exist (Farber *et al.*, 1992; Tienungoon *et al.*, 2000). The organism can survive and/or grow in brine solutions used during cheese manufacture, eg it survived for at least 132 days at 4°C in TSB containing 25.5% NaCl ($a_W = 0.785$) (Sutherland & Porritt, 1997). On Camembert cheese with a_W values of 0.959 and 0.984, listeriae populations declined during the first 20-30 days of ripening at pH 4.6-5.5 (Ryser & Marth, 1987b). Once the pH rose to 5.5 the *L. monocytogenes* grew indicating pH rather than water activity is primarily responsible for determining growth characteristics of *L. monocytogenes* in Camembert cheese.

The concern shown by the dairy industry over *L. monocytogenes* stems from a number of factors that make it more hazardous than other pathogens of potential concern. *L. monocytogenes* is ubiquitous in nature, is commonly found in food processing environments (Charlton *et al.*, 1990; Klausner & Donnelly, 1991; Cotton & White, 1992; Sutherland & Porritt, 1995; Fenlon *et al.*, 1996) and can grow at refrigeration temperatures (Walker *et al.*, 1990; Back *et al.*, 1993).

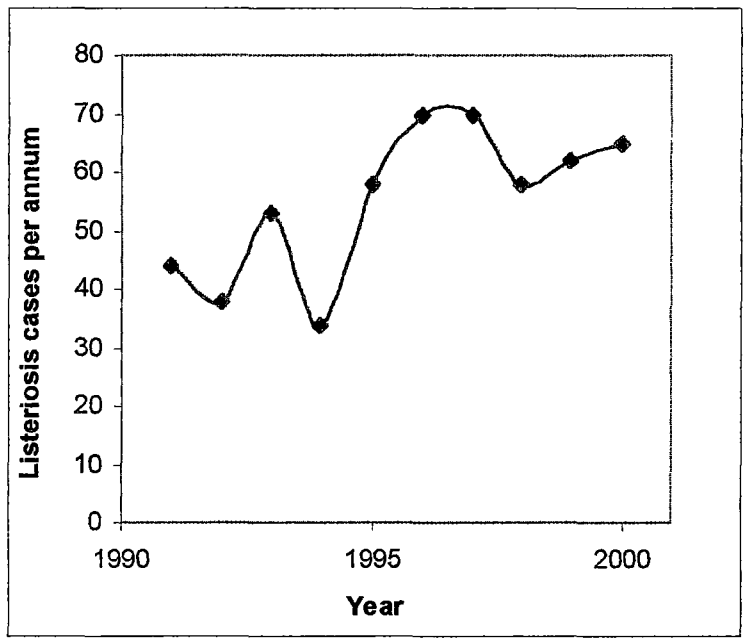
1.3.5.2 Prevalence of illness

Most listeriosis is believed to occur as sporadic cases, and this is supported by the number of cases of listeriosis which occur each year. In Australia the number of listeriosis cases reported to the National Notifiable Diseases Surveillance System is

around 50-60 cases per annum (Figure 1.2), the annual incidence estimated at 3 cases per million persons.

Figure 1.2 - Cases of listeriosis *per annum* in Australia 1991 - 2000

Data from Communicable Diseases Network - Australia New Zealand - National Notifiable Diseases Surveillance System (*pers. comm.*)



This is comparable to the incidence in many other developed nations, which is typically in the order of 2 to 5 cases per million inhabitants (Buchanan *et al*, 1997a). Data from the United States shows an annual incidence of ~0.7 case/million population, while in Canada there is an estimated annual incidence of 0.65 case/million people (Farber *et al*, 1996a). For European countries, a range of incidences was cited by Notermans *et al* (1998) as 0.1 to 11.5 cases per million people. The number of listeriosis cases in Tasmania during the period 1991-1999 was 9, an average of 1 reported case per year. The population of Tasmania was recorded as 459,659 in the 1996 census (Australian Bureau of Statistics, 1996), corresponding to an annual incidence of 2.2 persons per million, comparable to those figures quoted above, but slightly lower than the national average.

1.3.5.3 Hazard characteristation

Listeriosis is a severe, often fatal disease, and the milder symptoms are often described as 'influenza-like', including fever, convulsions, chills, headache, backache and also vomiting and diarrhoea. More complicated manifestations of the disease

include meningitis, septicaemia, encephalitis, and intrauterine or cervical infections in pregnant women which may result spontaneous abortion or stillbirth (Ryser & Marth, 1991). The incubation period before the development of the disease can be as long as ten weeks, and this has created considerable difficulty in determining the food implicated in the infection.

It appears certain members of the general population may be more susceptible to listeriosis (McLauchlin, 1995), with *L. monocytogenes* primarily affecting four subsets of the population: immunocompromised individuals, pregnant women, young children (including foetuses) and the elderly. However, sporadic cases involving apparently healthy people have been reported (Datta *et al.*, 1988; Azadian *et al.*, 1989; Ryser & Marth, 1991). The most concerning aspect of listeriosis is the fatality rate, which can be as high as 70% for untreated cases, but generally between 25 and 35%. Bean & Griffin (1990) demonstrated from bacterial pathogen epidemiological data that in terms of mortality rate, listeriosis is second only to tetanus in cases resulting in death, with a mortality rate of 31%.

It has been determined the most at risk products are ready-to-eat foods, including ready-cooked chicken, sliced ham, paté, processed meat paste, shellfish products, soft and surface-ripened cheese and foods held under refrigeration (Sutherland & Porritt, 1997). The proportion of the population who are considered vulnerable to listeriosis now form a considerable proportion of the population. Australian Bureau of Statistics show 12.3% of Tasmania's population is aged 65 years and over, compared to the national average of 12.1% (Australian Bureau of Statistics, 1996). Along with other susceptible groups, Buchanan *et al* (1997a) suggested up to 20% of the population may be vulnerable to listeriosis infection, and this has led to an increase in concern about *L. monocytogenes* as a foodborne pathogen. Highlighting this concern are several publications which have been made available to the public to warn pregnant women of the dangers of consuming the high risk foods outlined above, specifying soft cheeses such as Brie, Camembert and Ricotta (Health Department of Western Australia, 1995; National Food Authority, 1995)

1.3.5.4 Host susceptibility

Most people who become infected with *L. monocytogenes* are predisposed to the infection because of a disruption in their T cell-mediated immunity, with normal healthy adults making up less than 20% of all cases. This information tends to suggest the majority of people are resistant to infection with listeriosis (Ryser & Marth,

1991). Barza (1985) demonstrated approximately 70% of adult patients with listeriosis possessed some underlying immunosuppressive condition, or were using immune suppressing drugs such as corticosteroids. A small number of patients were alcoholics, accounting for 1 to 2% of all cases of bacterial meningitis in adults. Listeriosis was also shown to be a leading cause of bacterial meningitis in cancer patients (Burza, 1985).

Although the number of listeriosis cases in the general population appears to be small, the consequences of infection with *Listeria* can be very severe. Listeriosis is of little concern to healthy individuals and some individuals may carry the organism asymptomatically in the intestinal tract with carriage rates estimated to be about 5% in the normal population (Burza, 1985). Healthy people are able to inactivate the organism by virtue of cell-mediated immunity. To members of the susceptible population, listeriosis can result in a life threatening illness. The age distribution of reported cases is not uniform, but it appears most cases occur in the very young and the very old. Infants less than 1 year old comprise the largest group, and account for at least 25% of all cases. Approximately 55% of cases occur in adults older than 45, with 30% occurring in people over the age of 65, with most suffering from an underlying illness. The magnitude of the increased susceptibility due to the predisposing condition is reflected in the probability of infection. For the elderly, the risk of contracting listeriosis has been estimated at 1 in 40,000, for pregnant women, 1 in 8000 (Gellin & Broome, 1989). The risk among AIDS patients has been estimated to be 230 times that of the normal population (Jurado *et al.*, 1993).

1.3.5.3 Listeriosis outbreaks from cheese

Due to the long incubation periods associated with the disease, relatively few foods have been confirmed as causes of listeriosis outbreaks, however cheese has been one of the incriminated foods. Cheese contaminated with *L. monocytogenes* has a history of causing many cases of listeriosis over the past 15 years. The outbreak that served to raise awareness of *L. monocytogenes* as an emerging pathogen was in June 1985 in southern California, initially documented by James *et al.* (1985). The outbreak was linked to a brand of Mexican-style cheese and was ultimately responsible for at least 152 cases of listeriosis including 52 deaths (Linnan *et al.*, 1988) (Table 1.1). The severity of the outbreak considerably raised the profile of *L. monocytogenes* as a foodborne pathogen and prompted a great deal of research into the behaviour of the organism over the subsequent decade leading to the phrase "Listeria hysteria". Concerns were raised by regulatory bodies for the presence of the

organism in foods, particularly in soft cheeses, and many surveys were conducted into the incidence of *L. monocytogenes* on these products (Massa *et al.*, 1990; Greenwood *et al.*, 1991; Coveney *et al.*, 1994; Loncarevic *et al.*, 1995). The awareness generated by the 1985 outbreak also helped to alert authorities in Switzerland to an outbreak of listeriosis which had been taking place since 1983. By the time the source was finally discovered, (contamination of Vacherin Mont d'Or cheese with *L. monocytogenes*) the outbreak had resulted in at least 122 cases and 34 deaths (Büla *et al.*, 1995). *L. monocytogenes* was isolated from the cellars where the cheese was matured, as well as the wooden benches and brushes used during the ripening.

Other than these two well documented outbreaks, there have been several other sporadic cases of cheese-borne listeriosis. In England, a healthy nonpregnant 36-year-old woman developed meningitis during January 1986 after consuming a French-manufactured soft cheese 9 days previously (Ryser & Marth, 1991). Identical phage types of serotypes 4b were identified from the woman and an opened packet of the cheese. Several unopened packages were not found to contain the organism, suggesting the cheese may have become contaminated in the refrigerator, rather than during manufacture. This case highlighted the danger of cross-contamination.

Another case in February 1988, again in England, involved a healthy nonpregnant 40-year-old woman, admitted to hospital with "flu-like" symptoms (Azadian *et al.*, 1989). Identical phage types of serotype 4b were isolated from the woman and an opened package of Anari (Greek-style whey cheese) raw goat's milk cheese. The patient had consumed around 85g, and unopened packages were found to contain up to 5×10^7 cfu/g *L. monocytogenes*. A recall of the product was instituted, and a survey undertaken by McLauchlin *et al.* (1990) of products manufactured by the factory from which the cheese originated. It showed that 64% of retail cheeses and 50% of cheeses obtained directly from the factory were positive for *L. monocytogenes* 4b. The organism was isolated from shelving in the factory, leading to the conclusion that the cheese became contaminated during the final stages of manufacture and packaging. The calculation of McLauchlin *et al.* (1990), based on zero lag time and an initial load of 1-9 organisms/g, was that the generation time of *L. monocytogenes* on the cheese at 4°C was between 47 and 56 hours. This allowed the organisms to grow to high levels during the 3 month shelf life of the vacuum packaged product.

There are also other reports where cheeses were implicated but not confirmed as the cause of listeriosis. Ryser & Marth (1991) list these cases as:-

(a) isolation of *Listeria* from the blood of a 7-day-old infant in California whose mother consumed raw milk cheese 2 weeks before delivery;
(b) 3 cases of listeriosis in Arizona where the victims consumed a soft Mexican-style cheese;
(c) a possible association between listeriosis and consumption of an Italian cheese;
(d) one case of listeriosis in California where a woman delivered an aborted foetus after eating Monterey Jack cheese prepared from raw milk;
(e) an alleged Listeria abortion by a woman in New York who consumed contaminated Feta cheese;
(f) one case where *L. monocytogenes* 4b was isolated from a 3-year-old Washington state girl and cheese found in the family refrigerator
(g) isolation of an identical *L. monocytogenes* strain from a listeriosis patient in Philadelphia and from the cheese the victim reportedly consumed;
(h) one case involving a healthy woman from New Jersey who supposedly contracted listeriosis after consuming Ricotta cheese containing levels of *L. monocytogenes* of 10^6 cfu/g (Datta *et al.*, 1988);
(i) a case in Canada where *L. monocytogenes* serotype 1/2b was isolated from the blood of a 66-year-old man and from opened packages of imported soft cheese he consumed, as well as unopened packages (Farber *et al.*, 1990a).

Another listeriosis outbreak in 1995 was reported from Brie de Meaux soft cheese made from unpasteurised milk in France. At least seventeen people were affected including nine pregnant women. The infection resulted in two stillbirths, two abortions and one elderly person was put into a coma (Jacquet *et al.*, 1995). These outbreaks and case histories show *L. monocytogenes* has frequently been implicated in foodborne illness associated with cheese and justifies industry concern with this organism.

1.3.5.4 Behaviour of L. monocytogenes *in cheese*

The composition of many types of cheese products is favourable for the survival and growth of *L. monocytogenes*. Optimum conditions are found in cheeses with a pH greater than 5.5 and which do not contain starter organisms (Genigeorgis *et al.*, 1991). Back *et al* (1993) found fastest growth appeared to be in soft cheeses such as Brie, Camembert and Ricotta, whilst the slowest was in Cottage cheese. Back *et al*

(1993) found at 3°C there was no growth on Brie, but the organism still survived. A slight elevation in storage temperature allowed the organism to grow appreciably. A recommendation to store the cheese at 3°C was made, however the practicality of this is questionable. Commercial display cases and consumer refrigerators would normally run at temperatures higher than 3°C. As well, soft cheeses are most often eaten at room temperature, thus giving the organism an opportunity to multiply.

Genigeorgis *et al.* (1991) also found many cheeses did not support the growth of *L. monocytogenes*, including Blue, Cream, Swiss, Cheddar, Colby, Limburger and Feta. Under maturation conditions there was up to a 2 log decrease in numbers during storage. The harder type cheeses generally have low pH values, and are quite dry, conditions not very suitable for *L. monocytogenes*, with numbers tending to reduce during maturation.

1.3.6 Hazard identification

The hazard analysis has revealed *Listeria monocytogenes* is the main hazard that HACCP plans for the cheeses considered in this thesis should be concerned. The risk posed by this organism is greater than either *Salmonella* or pathogenic *E. coli* because it can survive in the factory environment, creating a higher risk of contamination and because it can grow during the shelf life of the products being studied. The mortality rate of listeriosis outbreaks is extremely high and it is more likely to contaminate cheese products as it can be present in the raw ingredient (milk), whereas it is less likely for *Salmonella* or pathogenic *E. coli* to be present.

The next stage in the implementation of a HACCP plan is to objectively identify critical points to control the hazard of *L. monocytogenes*, as well as institute the other elements of the HACCP system.

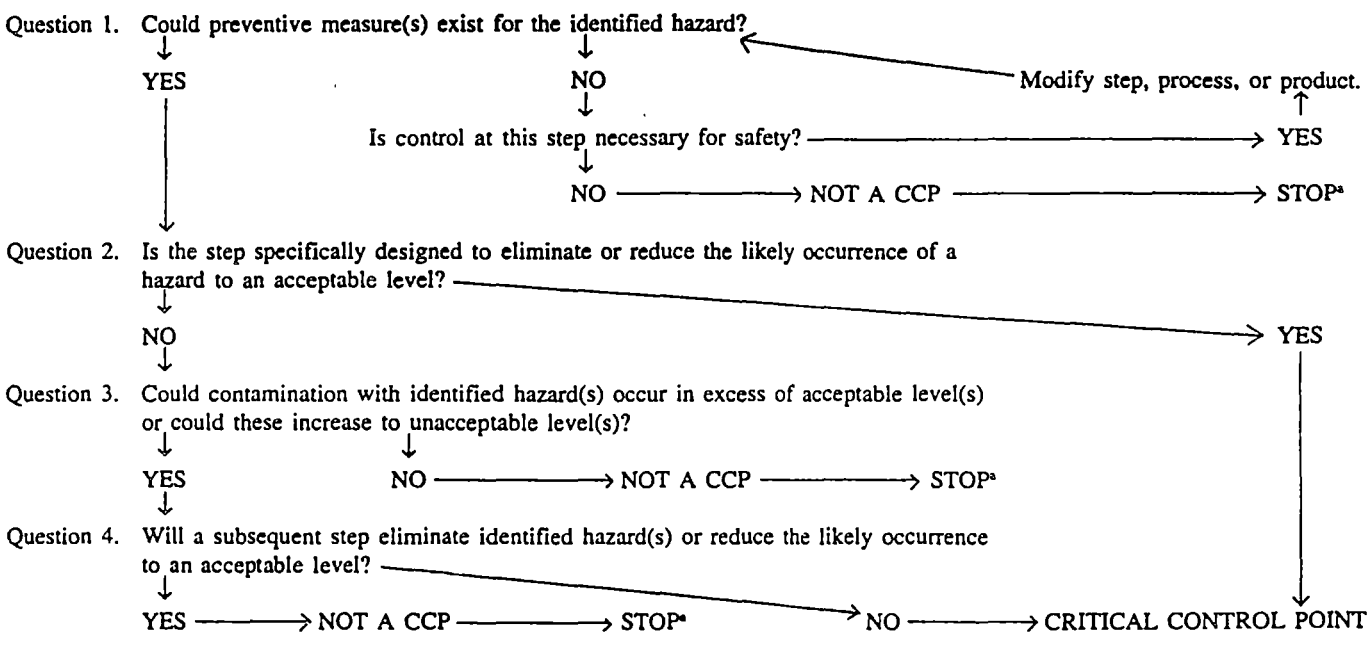
1.3.7 HACCP Principle #2 - Critical Control Points (CCP)

Once the hazard analysis has been conducted and the relevant microbial hazards of the process determined, the critical control points (CCPs) for those hazards in the product need to be identified. To achieve maximum success in food product protection, HACCP programs should be focussed on safety. CCPs should only be used to control those points in a food manufacturing process where lack of control will result in the development of a potential safety hazard. The standard definition of

a CCP is any point in the chain of food production from raw materials to finished product where the loss of control could result in an unacceptable food safety risk (Bauman, 1992). By including non-hazardous points, there may be too many control points to monitor, resulting in none being adequately monitored and jeopardising product safety.

The lack of quantitative information available has made the determination of CCPs a rather subjective process. To aid the decision making process, Decision Trees as shown in Figure 1.3 have been developed to help clarify what constitutes a CCP.

Figure 1.3 - Decision Tree for determination of a critical control point
from National Food Processors Association, (1993)



[a] Proceed to next step in the described process.

Despite decision-making aids being available, they still do not help to answer objectively the question of what constitutes an acceptable hazard level. There are a number of stages where CCPs may be needed to control the hazard.

Microorganisms can be destroyed or eliminated by thermal processing, or controlled by freezing and drying. After the microorganism has been eliminated, measures to prevent recontamination need to be taken. Alternatively, if the hazard cannot be totally eliminated from the food, microbial growth and toxin production must be inhibited. Growth can be inhibited through the intrinsic characteristics of the food, such as pH and water activity, or by the addition of salt or other preservatives. Conditions under which the food is packaged (aerobic or anaerobic) and storage temperatures (refrigeration or freezing) can also be used to inhibit growth.

Critical Control Points are required in distribution for time and temperature control. It is essential for the manufacturer to adequately chill products before they are loaded onto trucks for distribution since, in many cases, the refrigeration systems on trucks are designed to maintain temperatures, not to lower the temperature of the product. Exposure to high temperatures on loading docks needs to be limited in duration. Products which are sold within the state of Tasmania are not likely to be subject to long distribution times, due to the small distances. However, product exported interstate or overseas will undergo longer periods in the distribution chain and control needs to be exerted to ensure the product arrives in an appropriate state at the retail stage.

1.3.8 HACCP Principle #3 - Critical Limits

Once the CCPs of a process have been defined the next part of the HACCP implementation involves the establishment of critical limits which must be maintained at each point. Moberg (1992) describes a critical limit as "one or more tolerances that must be met to ensure a CCP effectively controls a microbiological health hazard. Critical Limits on CCPs represent the boundaries for safety". A CCP may have a number of factors which need to be controlled to assure product safety - for example, the pasteurisation step in the heat treatment of raw milk (flow rate, holding time, temperature), with failure to control all of these variables resulting in a loss of control of the process. The natural variability of the process must be accounted for, with the critical limit set below that at which a hazard may occur. Critical limits themselves may include confidence limits, so that the producer can estimate the level of risk to which they or their consumers are exposed by not taking action. There is subjectivity involved in deciding at what level to set the critical limit.

1.3.9 HACCP Principle #4 - Monitoring procedures

Monitoring provides an early warning that a process may be out of control but it is also done to collect data to subsequently provide information upon which to base a decision. Monitoring can be either observation (qualitative) or measurement (quantitative), the type required depending on the established critical limits for the CCP. Many factories already have in place monitoring programs for the purpose of product quality assurance. The in-house measurements may consist of time, temperature, pH, moisture and microbiological counts. These measurements can be applied in process modelling, which will be discussed in Chapter 3. Personnel must learn to take action immediately if anomalies appear in the process being monitored.

1.3.10 HACCP Principle #5 - Corrective action procedures

A HACCP plan needs to be incorporated into the operating instructions within a factory, so it is a working document. As pointed out by Tompkin (1992), it should not be a separate book placed on a shelf and forgotten until it is needed. Corrective action is the response taken when monitoring results indicate the operation is not under control. Action needs to be taken immediately should monitoring reveal a CCP is out of control. A number of activities are involved with corrective action including: use of the monitoring results to adjust the process to regain and maintain control; dealing with non-compliant product; correcting the cause of the problem and maintaining records of the corrective actions taken. Corrective action may include, e.g. the activation of the flow diversion valve in a pasteuriser, or extending the ripening time of a milk vat until the appropriate pH is reached. These actions are taken many times during the everyday operations of a factory, but are not necessarily recorded for later review. Any corrective action taken must be shown to bring the CCP back under control.

1.3.11 HACCP Principle #6 - Recordkeeping Systems

Records are an integral part of the HACCP plan as they are the only reference available to trace the history of a process, or a particular batch of product. Should a question arise over a product, the records kept by the factory may be the only way to ascertain or prove the product was prepared according to standard protocols. Recordkeeping may also allow an operator to recognise that a malfunction has occurred. Records are management's way to prove the procedures have been followed according to the HACCP plan. All HACCP records should be kept separate from quality assurance documents. If *L. monocytogenes* were detected on a batch of cheese,

then a recall would be necessary. Should this occur, effective recordkeeping allows personnel to trace back to the original ingredient batches, process data and possibly isolate the problem. Records should provide documentation for all ingredients, processing steps, packaging, storage and distribution.

1.3.12 HACCP Principle #7 - Verification procedures

Verification is an important step in the overall HACCP implementation, as it is used to demonstrate the system is in compliance with the plan. Verification confirms all hazards were identified in the HACCP plan when it was initially developed. Verification activities may include the review of the HACCP plan, CCP records, deviations, random sample collection and analysis, and written records or verification inspections. Product testing may be included (Kohn *et al.*, 1997). Verification is different from monitoring in that it is used to check the whole system is functional, rather than whether a CCP is under control. With proper implementation of a HACCP plan, end-product testing does not need to be emphasised.

1.3.13 Limitations of HACCP

The seven principles outlined above form the theoretical basis for the HACCP system, however, in reality the analysis of microbiological hazards and definition of CCPs and critical limits remains an inexact and subjective process. HACCP is frequently employed in a mainly qualitative fashion, since many fundamental questions cannot be answered quantitatively from current documented sources (Christian, 1994). To supplement the application of a HACCP system, predictive microbiology has been proposed as a quantitative method of describing the effect of process variables on microbial growth and inactivation and can be used to generate some of the necessary quantitative information (Baker, 1995; Ross & McMeekin, 1995; Elliott, 1996). A combination of predictive microbiology and HACCP offers the advantage of the systematic structured approach of HACCP towards tackling the problem at hand, with quantitative calculations at places where they are necessary. The link between predictive microbiology and HACCP will be demonstrated in Section 1.4.

1.4 Predictive Microbiology and HACCP

1.4.1 Predictive microbiology

It has become apparent in the study of food microbiology that food presents microorganisms with a nutrient rich environment (McMeekin *et al*, 1989). Thus, the

underlying hypothesis in the modelling approach is that nutrients will not limit microbial growth until spoilage has occurred or infectious dose levels are exceeded. Microbial behaviour is largely determined by a relatively small number of factors such as pH, water activity, temperature, atmosphere and the presence of organic acids (Roberts, 1997). Furthermore, the response of microorganisms to these environmental factors is a reproducible phenomenon (McMeekin *et al.*, 1993). Predictive microbiology is concerned with the quantification of microbial responses to the environment presented by foods, whether it be the specific growth rate, lag phase duration, the rate of population decline or the probability of growth being possible within a given environment. These responses can be summarised as simple predictive equations, which can be used to estimate the probability and amount of microbial growth that may take place.

The use of models in the food industry is not a new occurrence, with processes such as pasteurisation and canning based on models utilising D-values. Therefore the use of models for predicting growth of microorganisms was a natural progression, but has only occurred in the past two decades. Application of predictive models in the dairy industry is now becoming more widespread, but the full integration into HACCP implementation has had limited acceptance (Rowe, 1993; Griffiths, 1994; Murphy *et al.*, 1996). Once a detailed knowledge of the growth responses of a microorganism has been developed, predictive modelling allows a prediction to be made of the extent of microbial proliferation in the food during all stages of manufacturing, distribution and storage.

Two major types of modelling have been utilised in predictive microbiology, empirical and mechanistic. Empirical models simply describe the data in a convenient mathematical relationship, often giving no insight into the underlying process. Mechanistic or deterministic models are built up from theoretical bases, and can be interpreted in terms of known phenomena or processes. A monograph covering most aspects of predictive microbiology has been published (McMeekin *et al.*, 1993), as well as many review articles covering developments within the field (Davies, 1993; Whiting & Buchanan, 1994; McMeekin & Olley, 1995; Roberts, 1997; Walls & Scott 1997b).

1.4.2 The Modelling Process

A number of stages occur in the development of an appropriate model to describe bacterial growth (McMeekin *et al.*, 1993).

(i) **Planning:** Appropriate planning of experiments in order to make the model applicable over a wide range of conditions is necessary. Inoculum size and type, quantity of data readings needing to be taken, interactions between variables and whether to use food or laboratory media as the experimental growth medium all need to be considered.

(ii) **Data collection and analysis:** Methods for the enumeration of microorganisms can be either direct (viable count) or indirect (optical density, impedance, conductance), the method of choice dependent on time and budget constraints. Fitting of sigmoidal growth data also presents several choices in terms of which primary model to use (Section 1.4.2.1)

(iii) **Mathematical description:** Model parameters, and the effect of environmental factors on them, must be estimated using described techniques (Section 1.4.2.2).

(iv) **Validation and maintenance:** The model must be tested to evaluate how well it works in "real world" conditions, on the actual food product of interest (see Section 2 - Model evaluation).

1.4.2.1 Primary modelling

The observational method of fitting bacterial growth curves was very subjective. However, a number of sigmoidal growth functions are now available for this purpose. Nonlinear regression is used to generate objectively a sigmoid "curve of best fit" to growth data and the fitted equation used to estimate generation times and lag phase durations. One of the most commonly used sigmoidal growth functions is the so-called 'modified-Gompertz' function (McMeekin *et al.*, 1993), first proposed by Gibson *et al.* (1987) and shown in Appendix D (Calculations: Water activity, Generation time and Lag phase duration).

The modified-Gompertz function is an empirical application of a mechanistic model, which was initially derived for actuarial purposes. There is growing recognition that the modified-Gompertz may not be the most appropriate model to describe bacterial batch culture growth (Baranyi *et al.*, 1993; McMeekin *et al.*, 1993), but it is one of the simplest. Baranyi *et al.* (1993) developed a model which also described lag, exponential and stationary phases. Buchanan *et al.* (1997c) compared the Gompertz, Baranyi and a three-phase linear model for fitting bacterial growth curves and found all three described the data in a similar fashion. The Gompertz

model is a simple model, with few parameters. McMeekin *et al.* (1993) stated if several models give a similar fit to the data, it is best to use the simplest model. However, it has been found the Gompertz function tends to under-estimate generation times by ~13% (Baranyi *et al.,* 1993; Ross, 1993), and this must be taken into account if an equally good fit to the data, as other primary models, is to be obtained.

1.4.2.2 Secondary modelling

Square root model

The square root model was developed by Ratkowsky *et al.* (1982), initially for suboptimal temperatures only, and then extended to cover the entire biokinetic range Ratkowsky *et al.* (1983). This form of the temperature model has been combined with a square root water activity model, first proposed by Chandler & McMeekin (1989), later extended to describe the response of halophilic organisms (Miles *et al.*, 1997). A combined temperature and pH square root model was published by Adams *et al.* (1991), and the later modified and extended to include a term for lactic acid concentration (Presser *et al*, 1998).

Polynomial models

Polynomial models are another form of the empirical approach to modelling bacterial growth. These models have many more parameters than square root models and are usually constructed so they account for multiple factors. Buchanan & Phillips (1990) developed a polynomial model to describe the growth of *L. monocytogenes* which formed the basis of the model in the Pathogen Modeling Program (Buchanan & Whiting, 1994) (Appendix A – Equipment and Computer software).

Comparison between different forms of predictive models has been an ongoing debate within the microbiology community over the last decade, with argument over whether empirical or mechanistic models are more appropriate (Adair *et al.*, 1989; McMeekin *et al.*, 1989; Ratkowsky *et al.*, 1991; Zwietering *et al.,* 1991; Ross, 1993). Delignette-Muller *et al.* (1995) compared the accuracy of growth predictions from square root models and polynomial models for data from 14 published papers. They found polynomial models gave highly unsafe erratic predictions for generation time (GT), lag phase duration (LPD) and time for a 1000-fold increase. Average errors for GT and LPD were in the order of 35-40%, where the square root models gave highly unsafe predictions for LPD only, and had an average error of 11% for GT predictions and 36% for LPD.

1.4.3 Models in HACCP implementation

The concept of utilising predictive microbiology models to aid in the application of a HACCP system is not a new one. A number of review articles reinforcing the link have been published, with Ross & McMeekin (1995) describing the relationship as "almost intuitive". The link between the two ideas was mooted as far back as the early 1980's when Genigeorgis (1981) indicated predictive microbiology could provide a rational basis for drafting guidelines, criteria and standards to ensure the microbiological safety of food. Broughall *et al.* (1983) considered the concept of HACCP has often been applied to food processes without information about factors influencing microbial growth. The interactions of these factors have a significant influence on the application of predictive models into HACCP analysis of food processing and distribution practices.

While many articles continue to reinforce the link, until recently little work was reported in the area of monitoring processes within the factory environment and collecting the relevant data needed to incorporate modelling into the implementation of HACCP. Some work has been conducted on simulating temperature abuse conditions during the transportation and storage phases. Shellhammer & Singh (1991) investigated the use of time-temperature indicators to show possible temperature abuse of cottage cheese. Recent work by Zwietering & Hasting (1997a) presented a step by step approach to evaluating a food process in terms of microbial growth, either using a model for a particular organism, or a "super-organism", incorporating the fastest growth rates from different bacteria over the entire biokinetic temperature range, thus ensuring a worst-case scenario approach. Wijtzes *et al.* (1998) developed a decision support system integrating predictive modelling and process modelling in order to make predictions on food safety, thus empowering the HACCP system.

Generic HACCP plans can be adopted to aid in food safety implementation for smaller manufacturers. However, to ensure legitimate application of a HACCP system, each product needs to be considered individually, as each will contain its own inherent hazards. A number of scientific publications exist which outline the fate of *L. monocytogenes* during manufacture of specific cheese products: Semi-hard (Dominguez *et al.*, 1987; Bachmann & Spahr, 1995), Cheddar (Ryser & Marth, 1987a), Camembert (Ryser & Marth, 1987b), Feta (Papageorgiou & Marth, 1989a), Blue (Papageorgiou & Marth, 1989b), Swiss (Buazzi *et al.*, 1992), Mozzarella (Villiani *et al.*, 1996) and Afuega'l Pitu (Margolles *et al.*, 1997). This information is

valuable in the implementation of HACCP plans and, with the application of predictive microbiology, can assist in both initial implementation and maintenance of a HACCP system.

It has been well established there are four main areas where mathematical modelling of microorganisms can be used to assist in the application of a HACCP system. These stages include phases from the initial establishment of the plan: hazard analysis; establishment of CCPs; setting of critical limits; as well as the day to day operation of the plan, through ongoing monitoring of product parameters and determination of action should a lapse of control occur. It will be shown in Section 1.4.4 how predictive microbiology can be integrated into 4 of the 7 HACCP principles described previously.

1.4.4 HACCP Principle 1. Hazard Analysis

Hazard analysis involves determining which microorganisms pose a potential hazard to the food product. This is currently achieved by reviewing relevant literature (Bryan, 1996), to identify which organisms have caused problems with a particular product, or closely related products, in the past (Weingold *et al.*, 1994). However, many countries do not have foodborne disease surveillance programs, and those that do are generally inadequate due to funding restrictions (Todd, 1996). The accuracy of epidemiological data needs to be improved before it can be relied upon, as the majority of incidents still remain unreported. This data is also limited in that it is a reactive approach, offering no new insight to identify events which may happen.

There always exists the potential for a previously unencountered organism to present a problem. However, once an appropriate list of potential problem pathogens has been assembled, predictive models can be used to demonstrate just how much of a problem a particular organism may pose (van Gerwen & Zwietering, 1998). If a model shows the organism is not capable of growth on a certain food product, then it can be inferred a high level of contamination must occur before this will pose a problem (if the infective dose for the organism is high). However, if the model shows the organism can grow on the product, then it is obvious this organism poses a much greater hazard to the product, and any level of contamination could pose a problem.

Using an integrated approach it should be possible to identify organisms which may be expected to contaminate the product. Then, depending on the growth

rate of a particular organism, and how the environmental and intrinsic product parameters affect the growth (all of which can be quantified through models), it can be deduced which organisms pose the most serious hazard.

1.4.5 HACCP Principle 2. Determination of CCPs

Despite recent improvements in the area of rapid microbiological techniques, they are still impractical, in terms of time constraints, sensitivity and cost, for monitoring critical limits at critical control points. Therefore, fast indirect methods are required for continuous monitoring of the process, and it is here the value of predictive models becomes obvious. The monitoring of product parameters like temperature, pH and a_W is now relatively easy and fast, and is already conducted in many cheese factories. It has now been demonstrated that commercially available a_W meters can perform in a reliable and stable manner (Doe *et al.*, 1998), however, cost may put this technology out of the reach of smaller operators. Measured values can be entered into a predictive model to quantify the potential microbiological consequences of each individual handling/processing step, the likely effect on microbial germination, growth/toxin production, or death under the process conditions. The use of predictive microbiology allows the user to determine more objectively which steps are critical to product quality and safety and nominate them as the CCPs of the process.

Interpretation of a particular process step by survival/death models may reveal a particular process step results in significant decrease in the level of microbes of concern. Zwietering & Hasting (1997b) detailed how calculations for each process step can be made, once the parameters of time, temperature, pH and a_W are known. Certain steps in the process may show the time/temperature combination is such that there is little opportunity for microbial growth to occur. In this way, the entire process can be screened and the number of steps requiring more detailed descriptions can be identified.

Growth/no growth models (Ratkowsky & Ross, 1995; Presser *et al.*, 1998; Salter *et al.*, 2000; Tienungoon *et al.*, 2000) can also help to gauge whether a particular product formulation is well away from the growth/no growth boundary, ie. has a large safety margin to 'buffer' small variations in product formulation, and the product formulation represents a control point only. However, if the product formulation is poised just on the edge of the interface, it will be significantly affected

by small variations. Thus, in this situation, the product formulation will be a critical control point.

1.4.6 HACCP Principle 3. Establishing Critical Limits

The monitoring of the safety of processes by indirect measurements (ie. temperature, pH, a_W), rather than enumeration of microorganisms, is a theme common to both HACCP and predictive microbiology, and reinforces the concept that predictive modelling can be used to provide the quantitative information required to properly implement a HACCP system. The integration of the effects of these parameters over time, and their consequences for microbial growth, survival or death can be predicted using quantitative models.

It is possible to establish the dimension of critical limits that should be in place at CCPs by determining a number of "what-if" scenarios. Using predictive models it is possible to specify operating parameters which will prevent outgrowth; reduce the likelihood of germination or growth; limit proliferation to within acceptable limits; or achieve the required reduction in bacterial load. The combination of parameters which are able to achieve these aims can be seen on the computer screen without having to conduct a large number of trials. With probability models it is also possible to establish confidence intervals on the critical limits. The limits may refer to Minimum Inhibitory Concentrations (MIC's) or parameter combinations to ensure the reduction of the microbial load to safe levels, or to maximise time-temperature combinations which limit microbial proliferation to safe levels.

1.4.7 HACCP Principle 5. Corrective Action Specification

With predictive models it is possible to evaluate quantitatively the effect of losses of process control, and to make rational decisions regarding the fate of the product, eg. reprocess, lower grade of product, use in product subject to a more stringent thermal treatment. The information provided by predictive models also enables greater flexibility in the range of corrective actions which can be taken to achieve the same level of product safety, eg. by slightly modifying subsequent processing steps. If a rise in temperature in a coolroom occurs, the possible extent of microbial proliferation which occurs during the time period can be evaluated, and therefore the extent of the health risk to the consumer can be estimated.

1.4.8 Limitations

It is the quantitative part of the hazard analysis where predictive microbiology is very valuable. However, there is much uncertainty affecting predictions of bacterial lag times, imposing limitations on the usefulness of predictive microbiology, and leading to some reluctance on the part of industry to adopt the technology. Models to predict lag phase as a function of environmental parameters have been developed (three of which are evaluated in Chapter 2), but these predictions are reliable only if the prior history of the bacterial cells contaminating the product is known. In most cases it is not. One approach has been to adopt a worst-case scenario and assume no lag phase is present before the microorganisms commence growth (McMeekin *et al.*, 1993). But this leads to over-prediction of growth and, potentially, the rejection of perfectly safe food. Baranyi & Roberts (1994) demonstrated lag time is inversely proportional to growth rate, while Ross (1999) suggested lag time variability can be reduced through the concept of relative lag times or "generation time equivalents", ie. the ratio of lag time to generation time (LGR).

The use of relative lag times enables the effect of fluctuating conditions on lag time to be predicted, and to be included in risk assessment models for exposure assessment. From a practical perspective, a lag time of 3 generation time equivalents reduces the expected growth without lag by almost a factor of ten. In terms of critical limits imposed at a CCP, e.g. designating a limit to the extent of possible growth during a particular process to a specific number of log cycles, these differences due to lag times are very important. This concept is further discussed in Chapter 2.

1.4.9 Summary

HACCP and predictive microbiology can be viewed as complementary concepts, the latter enabling a more powerful use of HACCP by permitting objective and quantitative assessment of the effect of processing steps on product safety. Thus predictive food microbiology can be viewed as an extension of the HACCP concept, where a kinetic model can provide a direct measure of the stage and extent of microbial growth. Critical limits can be set from zero growth (lag phase not resolved) through any number of generations at which a public health or spoilage risk is perceived, and the appropriate measures taken to maintain the process within these limits.

Another emerging field in food safety is quantitative microbial risk assessment (QRMA), which incorporates some aspects of HACCP (Buchanan, 1995; Notermans & Mead, 1996) and is complementary to achieve the ends of food safety. QRMA attempts to quantify the probability of a consumer becoming sick from the eating the product. It is another area where predictive modelling can be used to further enhance food safety and will be reviewed in the following section.

1.5 Quantitative Microbial Risk Assessment

Quantitative Microbial Risk Assessment (QMRA) is the formal scientific process of identifying hazards and estimating risk. It attempts to provide a holistic view of the risks inherent in a process by modelling the process. Risk assessment is an area that can be incorporated into a HACCP system (Notermans & Mead, 1996), and also another area where predictive microbiology can be employed (Buchanan & Whiting, 1996; Foegeding, 1997; Walls & Scott, 1997b; Miles & Ross, 1999). Once the potential hazards have been identified, a risk assessment can be undertaken to define within an order of magnitude what risk of foodborne illness the product poses to the consumer. Risk assessment, is often considered to include four sub-elements (Buchanan, 1997):

(i) **Hazard identification**: requiring similar skills to those used in developing HACCP plans;
(ii) **Exposure assessment**: which requires the collection of demographics (Weingold *et al.*, 1994), food consumption patterns, use of predictive microbiology to assess the changes in numbers (Walls & Scott, 1997b) to assess, on average, how much a person is consuming;
(iii) **Dose-response relationship**: to relate the number of pathogens in the food to a "health outcome", data is usually taken from animal models or from epidemiological data;
(iv) **Risk characterisation**: the integration of all the information, typically using the techniques of mathematical modelling with simulation software.

The terminology within risk assessment has been confused on occasions, with the terms risk and hazard used interchangeably. A hazard is defined as the substance which will cause an adverse health effect, while risk is a combination of the likelihood of the hazard occurring multiplied by the severity of the adverse health effect.

1.5.1 Conducting a risk assessment

QMRA applied to foods is a concept still in its infancy with few examples of properly conducted risk assessments published in the scientific literature. One of the first microbial food safety risk assessments to successfully implement stochastic modelling (see Section 1.5.5.1) was that of Whiting & Buchanan (1997) for *Salmonella enteritidis* in pasteurised liquid eggs. This was followed by a QMRA for *Escherichia coli* O157:H7 in ground beef burgers by Cassin *et al* (1988a). However, the risk of microbiological food poisoning from cheese products has also been modelled, with van Gerwan *et al* (2000) conducting a risk assessment on the risk of *Clostridium botulinum* in cheese spread. Growth rates were based on theoretical processing conditions, with the outcome estimating the risk of botulism to be very low, equating to one death in a country the size of the Netherlands in 70 years. An exposure assessment for *L. monocytogenes* in cheddar-type cheeses in Canada was performed by Farber *et al* (1996a). Those authors utilised consumption data to estimate the number of potential listeriosis cases resulting from the consumption of these products. To date, the only QMRA for a cheese product which fully utilises stochastic modelling is that of Bemrah *et al* (1998). That study estimated the number of listeriosis cases (and resultant deaths), from the consumption of raw milk soft cheeses manufactured in France. Some of the assumption made by Farber *et al* (1996a) and Bemrah *et al* (1998) are discussed in the Methods and Materials Chapter (see Section 2.5).

The recently published risk assessment on *L. monocytogenes* by the USDA (USDA, 2001) provided one of the most detailed quantitative risk assessments undertaken for *L. monocytogenes*. The report featured quantitative risk assessments for a number of cheese varieties, among them soft mould ripened cheese and fresh soft cheeses. The exposure assessment was based upon two large-scale consumption surveys conducted in the United States, with the number of annual servings for each food category estimated. These figures are later compared with estimated consumption patterns generated from this thesis (Chapters 4, 5 and 6). The USDA report found that soft mould ripened cheeses had a moderate predicted relative risk of causing listeriosis on a per serving basis, while fresh cheeses presented a low risk, mainly due to the low number of annual servings (USDA, 2001).

One of the major limiting factors in conducting a risk assessment is the vast amount of information required to establish a health-related outcome at the

conclusion. Many of the proclaimed risk assessments conducted thus far have established an incidence of the hazard in the product, but failed to translate this into an outcome which can be interpreted as how likely the product is to cause foodborne illness. There are several examples of hazard assessments where the outcome estimates the probability of encountering the hazard in a product (Peeler & Bunning 1994; Van der Logt *et al*, 1997).

The data required to conduct a risk assessment includes establishing the contamination rate of the product by the hazardous organism. This may need to be estimated through information such as incidence of the organism in the factory and the conditions allowing it to survive or grow in this environment. If contamination does occur, the probability of the organism growing, its growth rate, the levels it can reach and how this will affect the consumer eating the product all need to be quantified. Data is required on the proportion of the general population which is most at-risk, consumption data to establish an exposure assessment, infective dose data and the end-use of the product. In most cases much of this data is lacking, therefore estimates need to be made during the course of conducting a risk assessment. It must be ensured any estimates made are realistic, and this should be tested in the final outcome. Usually, the final outcome of a risk assessment is based upon a combination of experience, epidemiological data, mathematical modelling and information in the technical literature.

1.5.2 Hazard identification

1.5.2.1 Raw milk quality

The quality of raw milk received by the cheese factory is crucial, not only for cheese quality, but also for product safety. For many products, raw milk handling is classified as a critical control point. On-farm practices can be used to minimise the risk of contamination, but this is usually out of the factory's control. Correct storage and delivery of raw milk is necessary to minimise growth of contaminating bacteria. The incidence of *L. monocytogenes* contaminated samples is generally low. Surveys indicate the worldwide prevalence of *L. monocytogenes* in milk to be about 2.2% (Sutherland & Porritt, 1997).

Australian survey results have shown a lower *L. monocytogenes* incidence. A New South Wales Dairy Corporation survey of 600 raw milk samples failed to detect *L. monocytogenes*, however, 0.4% of samples were positive for *Listeria* spp.

(Sutherland & Porritt, 1997). In their risk assessment of raw milk cheeses, Bemrah *et al* (1998) found the principal source of contamination to be environmental, with cases of *L. monocytogenes* mastitis rare. Hygiene and sanitation in the milking shed was found to be important in limiting the extent of contamination. Transport and holding facilities should protect the milk from contamination and be capable of maintaining milk temperature at 5°C or less (ANZFA, 1999).

1.5.2.2 Effectiveness of Pasteurisation on destruction of L. monocytogenes

Compulsory use of pasteurisation in Australia for all cheese milk does lessen the impact of on-farm practices on final product quality. The current Australian Food Standards Code (ANZFA, 1999) states "Milk and milk products used for cheese production shall - (i) be heat treated by being held at a temperature of not less than 72°C for a period of not less than 15 seconds, or at a temperature and for a period equivalent thereto in phosphatase destruction". However, raw milk storage must be effective in ensuring microbial growth does not occur to such an extent, that the effectiveness of the pasteurisation process could be exceeded.

Legislation governing manufacture of raw milk cheese varies in different states of Australia, but pasteurisation has been compulsory for all cheesemakers in Tasmania since 1993, prior to which, manufacturers producing less than 30 tonnes of cheese per year were permitted to make raw milk cheese (Willman, 1998). Previous concerns have been raised about the possibility of *L. monocytogenes* surviving pasteurisation due to the protective effect of cells occurring within bovine phagocytes. As a result, many publications have documented the effectiveness of pasteurisation on *L. monocytogenes* (Donnelly *et al* 1987; Doyle *et al*, 1987; Lovett *et al* 1990; Augustin *et al* 1998; Casadei *et al* 1998).

As appears to occur often with investigations into heat resistance of microorganisms, published reports concerning *L. monocytogenes* contain conflicting conclusions. Part of this is due to differing methodology used to determine heat resistance. Donnelly *et al* (1987) concluded the test tube method for evaluating heat resistance was inaccurate and *L. monocytogenes* cells dispersed freely in milk will not survive normal HTST pasteurisation. Bunning *et al* (1988) also refuted earlier reports that *L. monocytogenes* located within bovine milk phagocytes were more heat resistant. This was supported by Lovett *et al* (1990), who found populations of 10^5

cfu/mL freely suspended cells were inactivated by pasteurisation, as were cells inside bovine phagocytes.

Casadei *et al* (1998) found pasteurisation of high-fat dairy products contaminated with *L. monocytogenes* at 72.7°C for 15 seconds was capable of achieving a 7-12 log reduction (a mean D-value of 1.5 seconds). However, thos authors reported enhanced heat resistance when the organism was cultivated in dairy products. This increased heat resistance was not reported to be proportional to substrate fat content, but seen as linked to cell starvation due to cross-protective stress responses. Augustin *et al.* (1998) found applying a prior sublethal heat shock could increase thermal tolerance, with heat injury beginning above 45°C. The time required to kill 50% of the population was found to increase by up to 4 times when *L. monocytogenes* had been subjected to a prior heat-shock.

Lovett *et al* (1990) concluded when pasteurisation is carried out according to standard practices, then *L. monocytogenes* does not survive. The authors quoted an unpublished source of the highest $D_{71.7°C}$ value for *L. monocytogenes* in raw milk as 2.3 seconds, indicating a 6.5 log reduction of *L. monocytogenes* as the working capacity of a pasteuriser, similar to that reported by Casadei *et al.* (1998). Bradshaw *et al* (1991) reported a D-value of 0.9 seconds for *L. monocytogenes* in raw milk at 71.7°C, indicating pasteurisation was capable of a much larger reduction of *L. monocytogenes.*

Northolt *et al* (1988) assumed a concentration of *L. monocytogenes* at the factory to be one *L. monocytogenes* cell per 2 mL of raw milk. Even with prolonged temperature abuse, the authors calculated the highest concentration of *L. monocytogenes* presented to the pasteuriser would be in the order of $10^3 – 10^4$ cfu/mL. The conclusion of Lovett *et al* (1990) was that, even in a worst case scenario, a properly operated pasteuriser would be capable of inactivating *L. monocytogenes* in raw milk. To allow levels of *L. monocytogenes* contamination and growth to occur which could exceed pasteurisation effectiveness, raw milk handling would have to be uncontrolled. It therefore appears a logical step to state that pasteurisation is a CCP and raw milk handling is a Control Point (CP). From reviewed literature, it appears there is little likelihood of *L. monocytogenes* surviving HTST pasteurisation, and the most significant source of contamination is post-pasteurisation contamination.

1.5.2.3 Incidence - in factory

Data provided by Sutherland & Porritt (1995) can be used to assess the probability of the presence of the organism within the factory environment, and the potential risk of post-pasteurisation contamination. In their survey of 7 cheese factories in eastern Australia, 319 environmental swabs were collected, with 8% positive for *L. monocytogenes*. This study also demonstrated a dramatic difference in isolation rates between larger, export-oriented establishments and smaller manufacturers, which supplied the domestic market. None of the export oriented factories contained *L. monocytogenes* isolates, while the number of domestic factories positive for the organism were much higher; large domestic (17%) and small domestic (21%). This may be reflective of the availability of resources and capital for implementing HACCP and GMP programs. Smaller establishments may often lack resources, capital and technical expertise to implement food safety programs. In the large factories, programs are based on HACCP techniques and strict control of sanitation and hygiene. An earlier study had been conducted by the Victorian Dairy Industry Authority (Venables, 1989), concentrating on premises which manufactured "high risk" products. That survey detected *L. monocytogenes* in 19% of all environmental samples taken, corresponding to 40% (21/52) of the dairy factories surveyed. *L. monocytogenes* was also found in products from six manufacturers during the same study, two of these products were Ricotta.

The number of factories found to be *Listeria* positive in Australia appear to be greater than in studies conducted in the USA. Vasavada (1988) showed *L. monocytogenes* to be present in 19 out of 620 (3.1%) factories, with positives detected in finished product from 16 plants. A survey conducted in California for 156 milk-processing facilities showed *L. monocytogenes* to be present in 31 (19.9%) (Charlton *et al.*, 1990). For 41 cheese factories included in the study 2 (4.9%) factories were positive for *L. monocytogenes*. Pritchard *et al.* (1995) conducted a study on the incidence of *Listeria* on equipment and environmental sites within dairy processing plants. They found positive equipment isolates from 6 of the 21 plants (28.6%) and positive environmental isolates in 19 of the 21 plants (90.5%). Environmental samples included sites such as floors, drains and walls, and equipment samples included non-product contact surfaces of machinery and equipment. *Listeria* positive sites were found on equipment such as a cheese vat, pasteuriser, pasteurised milk holding tank, cheese turntable and two footbaths. The isolation from the latter site demonstrated proper maintenance of footbaths is essential to limit the spread of

the organism within the factory. Pritchard *et al.* (1995) concluded environmental contamination with *Listeria* does not necessarily translate into contamination of the equipment within the same plant. 2.9% of the finished product from these plants were contaminated with *Listeria* species. However, the presence of *Listeria* so close to finished product indicates the threat of post-processing contamination is very real. To estimate whether the incidence within the factory translates into product contamination, how well the organism survives within the factory environment needs to be established.

1.5.2.4 Survival on food and nonfood contact surfaces

L. monocytogenes is a very difficult organism to control in the food processing establishment, and moist cool environments allow it to survive. The presence of the organism on floors, drains, walls, ceilings, coolers and other locations within dairy factories demonstrate there is ample opportunity for this organism to contaminate dairy products after pasteurisation if the proper precautions are not carried out.

L. monocytogenes can adhere to food contact surfaces. The effect of temperature and pH on attachment to rubber and stainless steel surfaces was investigated by Smoot & Pierson, (1998a), and also following exposure to stress conditions (Smoot & Pierson, 1998b). The organism can attach very rapidly to stainless steel surfaces, readily to rubber surfaces and grow within biofilms (Jeong & Frank, 1994; Blackman & Frank, 1996). The possible pathways which may lead *L. monocytogenes* to contaminate the product and then establish itself to grow have been researched for the meat industry (Nesbakken *et al.*, 1996) and dairy industry (Sutherland & Porritt, 1995). Most reports indicate *L. monocytogenes* is a hardy organism and is relatively resistant to drying and low pH (Parish & Higgins, 1989; Klausner & Donnelly, 1991; Cotton & White, 1992; Jeong & Frank, 1994).Therefore, without effective cleaning and sanitation programs in place, the potential exists for the organism to survive within the factory environment and contaminate the product. Should contamination occur early within the cheesemaking process, numbers may increase within the cheese even if the organism does not immediately grow.

1.5.2.5 Concentration of bacteria within the curd

In a review of *L. monocytogenes* behaviour in cheese processing, (Pearson & Marth, 1990) stated that *Listeria* is mainly entrapped in the curd during manufacture

of most cheeses. Syneresis during the cheesemaking process causes the majority of bacteria to remain trapped within the curd, while whey drainage occurs. Studies with *E. coli* and *Enterobacter aerogenes* have shown a 10-fold increase in numbers occurs during curd formation (Kornacki & Marth, 1982), attributed by the authors to entrapment of organisms within curd particles. Ryser & Marth (1987b) did not observe this for *L. monocytogenes* during production of Camembert.

Bachmann & Spahr (1995) observed an increase in numbers of several pathogens in Swiss cheese curd over original inoculum levels and largely attributed this to physical concentration of bacteria by the curd syneresis. Reitsma & Henning (1996) found *E. coli* O157:H7 cells were more heavily concentrated in the curd during Cheddar manufacture than in whey. During manufacture of a Spanish acid-coagulated cheese, Margolles *et al* (1997) found *L. monocytogenes* was absent in whey samples, while counts in the curd increased up to 7 fold. During production of Feta, Papageorgiou & Marth (1989a) noted populations of *L. monocytogenes* recovered from whey represented less than 7% of the original inoculum (~3.6 log cfu/mL).

Tornadijo *et al* (1993) stated that increase in bacterial numbers in the curd could be partly explained by physical retention of the microorganisms in the curd, although bacterial multiplication must also contribute to this increase. Bemrah *et al* (1998) included in their model that 90% of bacteria were transferred to the curd, but the model was simplified in that no growth during manufacture was assumed. There are difficulties associated in differentiating the proportion of observed increase in bacterial numbers due to microbial growth, and that due to physical entrapment of bacteria within the curd. The latter will be a factor only when contamination occurs very early in the manufacture process, prior to hooping of the cheese.

1.5.3 Exposure assessment

There have been a large number of surveys into the incidence of *L. monocytogenes* in cheese products, largely attributed to awareness created by the 1985 Los Angeles outbreak, and subsequent isolations from imported French cheese in the USA. Large-scale surveys have been conducted overseas. In a survey of 333 cheeses produced in or imported into Sweden, *L. monocytogenes* was isolated from 6% of the samples (Loncarevic *et al.*, 1995), with raw milk cheeses more frequently contaminated with *L. monocytogenes* (42%) than cheeses made from heat-treated

milk (2%). Numbers on the product varied from <1 x 10^2 to 1 x 10^5 cfu/g, with no difference between pre-cut wedges and whole cheeses showing cross-contamination in the retail store did not play an important role. In an extensive 12 month survey conducted in the UK by the Public Health Laboratory Service (PHLS), Greenwood *et al.* (1991) reported *L. monocytogenes* was isolated from 63 out of 769 (8.2%) samples of soft ripened cows milk cheese, 13 samples of cheese containing more than 10^3 cfu/g, and two containing more than 10^5 cfu/g. Seven of those 13 were made from raw milk. In the same survey the incidence of *L. monocytogenes* in raw cows milk was 3.6% (13 from 361). A retail survey conducted by Hobson *et al.* (1991) in South Australia found no *L. monocytogenes* in 25 samples of soft cheese.

Hitchins (1995) used food consumption data and occurrence of foodborne *L. monocytogenes* for the period of the late 1980's to calculate the exposure rate of the human population to the organism. Using data from a survey reported by McLauchlin & Gilbert (1990), it was shown from 1130 samples of soft cheeses, 72 (6%) were reported as containing *L. monocytogenes*, with an average contamination rate of 404 cfu/g. Hitchins (1995) estimated the average intake of soft cheese to be 730 g per year, resulting in an estimate that *L. monocytogenes* would be ingested twice a year solely from soft cheese alone. The results of Teufel & Bendzulla (1994), presented in Notermans *et al.* (1998), indicated that a person consuming cheese 100 times in a year, eating 100 g per serve (an annual intake of 10 kg), would be exposed to levels of 10^3 or greater 0.7 times a year.

These values represent the presence of all *L. monocytogenes* strains, regardless of their pathogenic potential, therefore giving an overestimate of prevalence. It is well known that temperature abuse occurs quite often at the retail level and temperatures of 8°C are possible. Temperature abuse is likely to be partly responsible for the wide range of levels of *L. monocytogenes* observed in foods at retail level.

1.5.4 Dose response relationship

Due to the long incubation period of the organism, there have been very few opportunities to investigate incriminated foods. The source of infection has not been determined in any of the cases of listeriosis in Victoria (Ng & Forsyth, 1993). However, in the rare cases where the food product responsible has been traced, levels

of *L. monocytogenes* detected both from food remnants obtained from the patient or from unopened foods on retail sale have been high (>10^3 cfu/g) (McLauchlin, 1995). As stated by that author, much caution is needed in interpreting these results because of the small number of cases where information is available and the likelihood the infective dose will vary greatly between individuals. In outbreaks where cheese has been the incriminated food, the levels of *L. monocytogenes* detected in unopened food from the retailer has been between 10^3 - 10^7 cfu/g. Product samples taken during the 1985 Los Angeles outbreak routinely yielded at least 10^3 - 10^4 cfu/g of cheese.

Buchanan *et al.* (1997a) developed a dose-response curve for listeriosis from epidemiological and food survey data. This was done in a purposefully conservative manner and an ID_{50} of around 10^{10} was calculated for the general population (Chapter 2). Other estimates for the infective dose of *L. monocytogenes* from foods are typically greater than 10^3 (McLauchlin, 1995. Notermans *et al.* (1998) found quite high oral doses were required to be given to mice before the onset of disease. Even for immunosuppressed nonprotected mice the ID_{50} was 6.3 $\log_{10}$ organisms. They proposed the protection offered by the intestine is unaffected by the underlying immune system, with immunologically protected mice possessing an even higher ID_{50}. Therefore two components contribute to the protection of humans against infection with listeriosis; a nonadaptive response offered by the physical status of the intestinal barrier, and an adaptive response of the immune system. For disease to occur there needs to be a number of simultaneous events; a large number of *L. monocytogenes* to be present in the product, the intestinal layer needs to be breached and the immune response is delayed (Notermans *et al.*, 1998). The need for all these events to occur in order for listeriosis to occur, provides an explanation of the low incidence of listeriosis in the community in comparison to the food-borne exposure which is estimated to be high.

Considering survey results of the incidence of *L. monocytogenes* in foods, it appears consumption of small levels of *L. monocytogenes* (10 cfu/g) may be widespread in the general population (Hitchins, 1995; Notermans *et al.*, 1998). Prevalence of the organism in foods suggests exposure rates to the organism are relatively high, but the incidence of disease also suggests the probability of contracting the disease is low.

1.5.5 Risk Characterisation

In the preceding discussion all the elements necessary to undertake a risk assessment were introduced. Those elements will be combined with stochastic modelling principals to complete the risk characterisation step.

1.5.5.1 Stochastic Modelling

Traditional risk assessments have used mathematical models to estimate risk as a function of one or more inputs, and typically relied on point estimates, such as an analysis determining the "worst case", "best case" and "expected" outcomes. However, by choosing single numbers for inputs, risk assessors unavoidably ignored the uncertainty and variability in the risk estimate. All food processes are variable, and the events which affect the safety of the product are also variable. This variability needs to be taken into account, as a calculation of outcomes based on average values for each variable will ignore unusual, but highly important outcomes. An option which has been used more in recent times is "Monte Carlo"-type stochastic modelling (Vose, 1996), where point estimates are replaced with probability distributions.

Each input variable within a process has a range of values it can take, which can be described by a distribution function (normal, uniform etc). Stochastic modelling involves running through the model numerous times, each run being called an iteration. With each iteration, a value is selected from each variable range (the frequency dependent upon the type of distribution applicable to that variable) and the outcome is evaluated for that set of circumstances. The software performs many iterations to determine what the probability of each outcome is from the combination of variables, and a "picture" of the range of possible outcomes is generated. The advent of commercially available computer programs to automate this process, such as @RISK (Appendix A), has made stochastic modelling methods one of the key techniques of quantitative risk assessment. The software can perform tens of thousands of iterations in a few minutes to generate a spectrum of possible outcomes, and from this can determine the range and likelihood of occurrence for each possible outcome. This includes the unexpected and undesirable outcomes, which although they may be rare, could prove to be extremely important to the health of the consumer. Further analysis of the model provides information about the key inputs most significantly influencing the risk outcome, thereby identifying potentially effective intervention strategies. Manipulation of the model, by altering input values

in "what if" scenarios, can readily provide insight into the effectiveness of proposed risk intervention strategies.

1.5.5.2 Sensitivity analysis

When conducting a risk assessment it needs to be understood how all the factors and their variations interact to influence the range of possible outcomes. Stochastic modelling software can conduct a sensitivity analysis, which records the relationship between the magnitude of the input variable and the size of the output. Sensitivity analysis determines the sensitivity of each output variable to the input distributions in the worksheet, and identifies the most "critical" inputs in the model. The @RISK software performs the sensitivity analysis using rank correlations, based on the Spearman rank correlation coefficient calculations (Vose, 1996). With this analysis, the rank correlation coefficient is calculated between the selected output variable and the samples for each of the input distributions. The higher correlation between the input and the output, the more significant the input is in determining the output's value. The Spearman rank order correlation coefficient (ρ) is calculated as shown in Eqn 1.1.

$$\rho = 1 - \frac{6\Sigma(x_i - y_i)^2}{n(n^2 - 1)} \tag{1.1}$$

x_i and y_i are the ranks of the ith pair of the two variables X and Y.

Rank order correlation is a non-parametric technique for quantifying the relationship between the two variables. Therefore, unlike least squares regression, which requires the relationship to be linear, rank order correlation is not affected by the type of mathematical relationship between the variables. The technique does not require an identification of which variable is dependent and which is independent as the calculation for ρ is symmetric. The value of ρ varies from -1 to 1 in the same way as the least squares regression coefficient (r). A value of ρ close to -1 and 1 means the variables are highly negatively and positively correlated respectively. A value of ρ close to zero means there is no correlation between the variables. A 'tornado plot' can be developed which allows the user to visualise which are the "high-risk" input factors, those which strongly correlate to the size of the output, providing an objective method of rating the most important factors contributing to risk. In this manner, the

risk assessment can be used to identify potential Critical Control Points. The risk assessment process must be conducted individually for each product, however, there are a number of elements common to the cheesemaking process which can be considered on a collective basis.

1.5.5.3 Limitations

One aspect of conducting risk assessments which must be understood is that the simulation model and outputs are limited by the quality of data which is included in the original equation. One of the main problems in the area of risk assessment, and the main determinant of why so few authentic examples exist, is the lack of quality data. This has lead to most quantitative risk assessments using estimations for some variables, thus leading to semi-quantitative outcomes. In reality, this may be the situation for some time to come, as some data for the assessment are very difficult to gather, such as pathogen dose-response (Coleman & Marks, 1998). There is considerable cost involved in generating sufficient data to establish a fully quantitative risk assessment, leading to the use of published data and estimates wherever possible. Vose (1996) stated one of the most important considerations when conducting a risk assessment is that each iteration must deliver a scenario that is potentially observable in real life. The model must be restricted to prevent it from producing, in any iteration, a scenario that could not physically occur. Miles & Ross (1999) referred to the GIGO syndrome (Garbage In, Garbage Out), pointing out a modelling system is only as good as the data the user provides it with. The sensitivity analysis also allows the consequence of any estimates which were made during the risk assessment to be visualised. By making small changes in these assumptions, it can be seen if this has a large impact on the final result, thus cautioning the user about the accuracy of the assumption.

1.6 Summary

The three elements of predictive microbiology, HACCP and risk assessment are all complementary with the general aim of providing safe food to the consumer. The integration of these principles within the food industry is still in its infancy, and one of the objectives of this thesis is to establish a protocol for data collection and the integration of predictive and stochastic modelling into HACCP implementation. This has the potential to empower the HACCP system, removing much of the subjectivity involved with HACCP, and supplying quantitative data to enable objectivity in determining critical control points, critical limits and corrective action. This will be

conducted for three specialty cheeses, produced in factories varying from small domestic operations to larger export-oriented factories. The first stage in this implementation is to select an appropriate model to predict the growth of *L. monocytogenes*, then a risk assessment will be performed on each of the cheese products in turn.

2. MATERIALS AND METHODS

2.1 Cheese products

Specialty cheeses were selected for this study primarily because the products provide favourable conditions for bacterial growth. Priority was given to products that were previously identified in published literature as being associated with outbreaks, or supporting the growth of *L. monocytogenes*. With assistance from the TDIA, a survey of Tasmanian specialty cheesemakers was undertaken to identify factories manufacturing suitable short shelf-life products.

As demonstrated in Chapter 1, surface-ripened cheeses such as Brie can support the growth of *L. monocytogenes* (Genigeorgis *et al.*, 1991; Back *et al.*, 1993) and have been responsible for several listeriosis outbreaks (Büla *et al.*, 1995; Jacquet *et al.*, 1995). Brie is produced in a variety of sizes, however a 1 kg package size was chosen for this study due to a higher moisture content that provides more favourable conditions for microbial growth than smaller-sized Brie. Whey cheeses including Ricotta have also been implicated in sporadic cases of listeriosis (Datta *et al.*, 1988; McLauchlin *et al.*, 1990) and also readily support the growth of *L. monocytogenes* (Genigeorgis *et al.*, 1991; Davies *et al.*, 1997). Cases of listeriosis linked to Cream cheeses have not been reported, however Mascarpone was identified as the cause of a fatal botulism outbreak in Italy (Anon, 1996). Therefore, as conditions exist in all of these cheeses for growth of pathogenic bacteria, the potential exists for each to pose a hazard to the consumer. Despite the previous detection of *L. monocytogenes* in a batch of shredded cheese (Fig. 1.1), this product was not included in the study as it has been shown that the hard cheese types used for shredded cheese do not support *L. monocytogenes* growth (Ryser & Marth 1987a; Ryser & Marth, 1999).

2.2 Monitoring of cheesemaking parameters

Chapter 1 highlighted that a lack of food process data may explain the failure of predictive modelling to be fully utilised in HACCP implementation and risk assessment systems. As insufficient factory process data was available for the cheese products studied here, data collection was required to allow full characterisation of the cheesemaking process and establishment of parameter values and mathematical distributions for stochastic modelling.

The cheesemaking process was monitored within the factory environment, commencing from the end of heat treatment when the temperature of the product cooled to 40°C. The number of production runs monitored was 8 for Ricotta and Mascarpone, and 10 for Brie production. It was assumed that all *L. monocytogenes* would be inactivated by the HTST pasteurisation process (Section 1.5.2.2), and that any growth would be due to post-pasteurisation contamination. It was assumed that any contaminating organisms would not grow until the milk / curd temperature reached 40°C, as Augustin *et al.,* (1998) showed heat damage of *L. monocytogenes* commences at 45°C. The process was monitored through to packaging, coolroom storage and distribution. Temperature, pH and a_w were measured, and a microbiological profile established. Monitoring bacterial numbers during the manufacturing process allowed potential contamination sources to be highlighted. When factory data was available, process data from the previous 12 months was used for further refinement of estimates of process variability.

2.2.1 Temperature

Process temperatures were monitored at 3 minutes intervals with Tiny Tag temperature data loggers (Appendix A – Equipment and computer software). The temperature probe was placed into the milk/cheese and followed for an entire production run. All temperature profiles were exported to a Microsoft Excel® (Appendix A) spreadsheet for subsequent modelling of *L. monocytogenes* growth. Temperature profiles for storage and distribution stages were measured by placing a temperature logger inside a cardboard box, with the probe wedged between two packaged products. This box was included within a pallet of the product at a random position and collected from the factory for commercial distribution.

2.2.2 pH and water activity determination

Ten gram samples were aseptically removed from the cheese and placed into sterile specimen containers. Sampling equipment was soaked for a minimum of 15 minutes in quaternary ammonium sanitiser solution prior to use. pH was determined (Orion Model 250A pH meter, with calomel sealed flat tip probe; Appendix A). Readings were obtained by touching the surface probe directly onto the cheese, and rinsing the probe with distilled water between readings. Where two phases were present, for example Ricotta which tended to have liquid within the package, the pH of both phases was determined.

Water activity was measured using an Aqualab Model CX2 water activity meter (Appendix A). The meter was switched on 30 minutes prior to use to allow the meter to equilibrate to room temperature, as per the manufacturer's instructions. The meter was calibrated with distilled water and a saturated NaCl solution prior to measurement. Five readings were taken for each sample, and the mean value calculated.

Some *L. monocytogenes* predictive models calculate growth predictions based on salt concentration, which required the conversion of a_W measurements. This was achieved using the conversions listed in Appendix D. The pH and a_W values were transferred to an Excel spreadsheet and aligned with corresponding temperature measurements. Interpolation of values between measurements of pH and a_W values was necessary because a_W and pH readings were made less frequently than temperature. A uniform rate of change of pH and a_W was assumed to avoid large "jumps" in pH and a_W values during subsequent stochastic modelling. On occasions where values of pH or a_W did change rapidly, sampling was conducted more intensively to more accurately describe this.

2.2.3 Microbiological examination of cheeses

A microbiological profile of the cheesemaking process was performed (Standard plate count, Lactic acid bacteria, Yeasts and Moulds), with 10g samples removed using the same method used for pH and a_W samples (Section 2.2.2). Final product attributes were assessed by purchasing cheeses from a local distributor, or direct from the factory when possible. Thus the cheese was not subject to any temperature abuse that may potentially occur from retail and consumer handling. The product was aged between one and three days from date of packaging when sampled. All samples were kept refrigerated until the analysis could be conducted (usually within 24 hr).

All microbiological examinations were conducted according to Australian standard methods (Appendix B – Australian Standard Methods). All cheese samples were prepared according to AS1766.3.15, and were homogenised in 90 mL mixture of 2% trisodium citrate plus 0.1% peptone solution in a stomacher for approximately 3-4 minutes. The homogenate was serially diluted and appropriate dilutions, prepared according to AS1766.1.2, were used to enumerate the microorganisms listed below.

2.2.3.1 Standard Plate Count (SPC)

Standard plate counts (SPC) were carried out using pour plates when numbers of bacteria were expected to be below 10^2 cfu/g (AS1766.1.3) or spread plates for higher numbers (AS1766.1.4). Standard plate count agar (Oxoid CM463) was used and incubated at 25°C for 24-48 hr.

2.2.3.2 Lactic acid bacteria

Lactic acid bacteria were enumerated by spread plating 0.1 mL of appropriate dilutions on M17 agar (Oxoid CM785). Plates were incubated at 35°C for 48 hr.

2.2.3.3 Yeasts and moulds

Yeasts and moulds were enumerated by spread plating 0.1 mL onto Oxytetracycline Glucose Yeast Extract agar (Oxoid CM545 + Oxoid SR73) according to AS1766.2.2. Plates were incubated at 25°C for up to 4 days and yeast and mould colonies counted separately.

Ten samples of each cheese type were sampled to identify if other contaminating organisms were present in the cheese products. These tests were performed according to methods outlined below.

2.2.3.4 Coliforms

Coliform enumeration was performed according to AS1766.2.3, using the Violet Red Bile Agar (Oxoid CM107) pour plate method with overlay. Plates were incubated at 35°C for 24-48 hr.

2.2.3.5 Escherichia coli

Examination of cheese samples for *E. coli* was conducted by spread plating 0.1 mL of appropriate dilutions of the homogenate onto Eosin Methylene Blue (EMB) agar (Oxoid CM69), and incubated at 30°C for 24-48 hr.

2.2.3.6 Salmonellae

25g samples of cheese were examined for salmonellae following AS1766.2.5. Each 25g sample was placed in lactose broth as a pre-enrichment step, and incubated at 35°C for 16 hr. 1 mL samples were transferred into 10 mL volumes of Tetrathionate broth (Oxoid CM29) for selective enrichment at 35°C. Samples were streaked onto Brilliant Green Agar (Oxoid CM263) and XLD medium (Oxoid CM469) after 24 and

48 hr to differentially select for salmonellae. These plates were incubated at 35°C for 24 hr.

2.2.3.7 Staphylococcus aureus

Examination for *S. aureus* was conducted according to AS1766.2.4, involving plating on Baird-Parker agar (Oxoid CM275) + Egg yolk Tellurite emulsion (Oxoid SR54). Plates were incubated at 35°C for 24 hr, with negative plates then re-incubated for a further 24 hr.

2.2.3.8 L. monocytogenes

25 g samples of cheese were tested for the presence of *L. monocytogenes*, according to the interim standard method (AS1766.2.15). 225 mL of Listeria Selective Enrichment Broth (LEB - Oxoid CM862 + Oxoid SR141) was added to the cheese, and homogenised for 4-5 min. This was incubated at 30°C and sub-cultured by direct plating onto Listeria Selective Agar (LSA - Oxoid CM856) using the 16 streak technique after 1, 2 and 7 days. A positive control was included to identify typical *L. monocytogenes* colonies.

Australian standard AS1766.2.15 is an interim method from 1991. Therefore, it was deemed necessary to evaluate the sensitivity of the method in the cheese products being studied by inoculation with low levels of *L. monocytogenes*. This was completed (see Appendix B) and it was shown that the sensitivity of the standard method is satisfactory for the needs of the current study, with *L. monocytogenes* detected at very low levels.

2.3 Product challenge tests

Challenge tests with *L. monocytogenes* were conducted on cheese products at temperatures of 5°C and 20°C, chosen as typical refrigeration and abuse temperatures respectively. Packaging conditions were simulated, i.e. vacuum packaging for Ricotta and Mascarpone. The products were also inoculated under aerobic conditions to mimic opening of the packages during consumer handling of the product. Dalgaard & Jørgensen (1998) suggested that naturally contaminated product should be used for challenge tests, as this is more reflective of the real-life situation. They demonstrated growth of *L. monocytogenes* in naturally contaminated product may be slower than growth in inoculated challenge tests. However, since no naturally contaminated cheese samples were found in the present study, inoculated product was used for all model evaluation studies.

2.3.1 Preparation of cheese and inoculum for challenge tests

2.3.1.1 L. monocytogenes strain

A wild-type *L. monocytogenes* strain was used in the validation studies and prepared according to the methods presented in Appendix C (Culture maintenance and preparation of inoculum). The culture was maintained deep frozen by the method outlined in Appendix C.

2.3.1.2 Aerobically stored product

200g samples of Mascarpone and Ricotta cheeses were aseptically placed into blender bags (Appendix A). Growth experiments on Brie curd involved removing the outer 2-3mm of the Brie mould surface with a sterile knife, and placing 200g of the cheese curd placed into a blender bag. 1 mL of the prepared inoculum was added to the cheese, resulting in a final inoculum level of ~10^4 cfu/g. Homogenous distribution of the inoculum was achieved by stomaching the cheese for 4-5 min. The bag was taped shut, but not airtight, wrapped in a watertight bag and then placed in a refrigerated waterbath (Appendix A) to ensure correct incubation temperatures were maintained. A weight was placed on top of the whole package to ensure it remained submerged in the water.

The wrapping was removed from a sample of Brie. Prepared inoculum was transferred to a sterile beaker, and a paintbrush used to apply the inoculum to the mould surface of the cheese. The brush was covered entirely with the inoculum, and dabbed against the side of the beaker to remove excess liquid. The inoculum was spread over the cheese surface to obtain an even coating, on the top surface and the side. The cheese surface was allowed to dry for approximately five minutes, then wrapped back into the original wrapper, placed into a watertight bag and placed in a waterbath at the appropriate temperature, as described above.

2.3.1.3 Vacuum-packaged product

For vacuum-packaged cheeses, the cheese was bought in commercially available packages of 1 kg. These were then opened and the cheese aseptically subdivided for convenience into 20g portions and placed into small barrier bags (Appendix A). The inoculum (0.1 mL of ~10^5 cfu/g) was added. The bags vacuum packed and sealed (Appendix A). The cheese packages were submerged in boiling water for 2-3 sec to heat shrink the bags to simulate packaging procedures in the factory. A quantity of the packaged cheeses was placed in a larger bag and was submerged in a waterbath at the appropriate temperature, as described earlier.

2.3.2 Enumeration of *L. monocytogenes*

Initially both non-selective (Tryptone Soya Broth with 0.6% Yeast Extract TSB-YE) and selective (LSA) agars were used to enumerate *L. monocytogenes* from the cheese. Enumeration with non-selective media was made difficult by competing biota. Therefore LSA was used for enumeration of *L. monocytogenes* in all cases. Two types of diluent were compared for enumeration, that used by Ryser & Marth (1987b), 0.1% peptone water with 0.1% Tween 80 and cheese diluent prescribed in the Australian Standards (AS1766.3.15). Addition of Tween 80 did not significantly improve detection (data not shown), therefore the Australian standard cheese diluent was used.

At predetermined sampling times, bags were removed from the water bath. In experiments where the cheeses had been individually packed, the bags were sprayed with ethanol and cut open with sterile scissors. A 10g sample of cheese was aseptically removed with a sterile spoon and placed into a blender bag. 90 mL of diluent was added and the mixture stomached for 2-3 min. 1 mL of the resultant suspension was serially diluted to appropriate levels and 0.1 mL spread plated onto LSA and incubated for a maximum of 48 hr at 25°C. Plates containing the appropriate number of colonies (30-300) were counted and total numbers calculated according to the method of Farmiloe *et al.* (1954). Average growth rates and lag phases were calculated by the modified Gompertz Equation (Appendix D).

2.4 Stochastic modelling of the cheesemaking process

A stepwise procedure for conducting a risk assessment was presented by van Gerwen & Zwietering (1998). The authors' suggested undertaking an initial 'rough' or semi-quantitative risk assessment, to assess orders of magnitude for microbial processes through the use of simple models. This can be done for microbial growth by assuming first order kinetics and neglecting lag phase and stationary growth. The resulting prediction is a worst-case scenario, that can be refined through subsequent addition of data to provide a more accurate outcome.

The performance of a 'rough risk assessment' provides an efficient method for quickly establishing the main determinants of risk in a process. Risk determinants may be quantified by comparing several models, but van Gerwen & Zwietering (1998) cautioned variations in food processing parameters may affect outcomes of a risk assessment more than the choice of the predictive microbiology model. In Chapter 3,

effectiveness of predictive models for prediction of *L. monocytogenes* growth on cheese products is demonstrated, and a model selected to generate stochastic modelling outcomes.

2.4.1 Modelling strategy

Following a strategy similar to that of van Gerwen & Zwietering (1998), a simplified semi-quantitative risk assessment was initially conducted for each cheese product. This was termed a process risk model (PRM), after the approach of Cassin *et al.* (1998), as it allowed estimates of potential *L. monocytogenes* growth during each process step to be made, and a sensitivity analysis was performed to correlate the parameters (temperature, pH, salt concentration) which contributed to the worst outcomes. The Process Risk Model did not include estimates of lag phase, and was not used to generate an estimate of listeriosis risk. Conducting this initial assessment is valuable in locating the most important risk-contributing factors within the process.

The second, more detailed modelling stage involved the addition of parameters to perform a quantitative risk assessment of the probability of the cheese products causing listeriosis. Estimates were obtained for many of these additional parameters. The data were analysed using BestFit® to determine the distribution function that best described the variance in the data. In many cases the variable was shown to follow a normal distribution, or to be at least as well described by a Normal distribution as any other (Fitting statistics shown in Appendices E-G). Where insufficient data were available a Triangular distribution was selected (Tables 2.1-2.2). Vose (1996) stated the Triangular distribution is often considered to be appropriate where little is known about the variable outside an approximate estimate of minimum, most likely and maximum values. The parameter estimates were made specific for each cheese product, and justifications for these estimates are discussed later in this Chapter and in individual Case Studies (Chapter 4-6). Tables 2.1 and 2.2 detail the distribution functions identified or selected. Input parameters requiring derived estimates from model outcomes (eg. Dose), or those derived from literature data (eg. probability of infection) are shown in Table 2.3

The @RISK software (Appendix A) was used to run all stochastic modelling simulations, with the minimum number of iterations set at 10,000 for each scenario tested. Convergence was monitored for all outputs to ensure that the criterion was met (i.e. convergence was achieved if the percentage change of all outputs was less than 1.5%).

Table 2.1 - Description and distribution of input variables for detailed quantitative risk assessment to end of coolroom storage

Variable	Description	Unit	Distribution / Model
Production Temperature	Temperature measured during production	°C	Normal (mean, standard deviation)
Production pH	pH measured during production	-	Normal (mean, standard deviation)
Production salt concentration	Salt concentration calculated from measured water activity during production	%	Normal (mean, standard deviation)
Draining Temperature	Temperature measured during draining	°C	Normal (mean, standard deviation)
Draining pH	pH measured during draining	-	Normal (mean, standard deviation)
Draining salt concentration	Salt concentration calculated from measured water activity during draining	%	Normal (mean, standard deviation)
Maturation Temperature	Temperature measured during maturation	°C	Normal (mean, standard deviation)
Maturation pH	pH measured during maturation	-	Normal (mean, standard deviation)
Maturation salt concentration	Salt concentration calculated from measured water activity during maturation	%	Normal (mean, standard deviation)
Log start number	Number of *L. monocytogenes* contaminating cheese	Log cfu/g	Triangular (-3, -1, 3)
Time of contamination	Time during manufacture when cheese is contaminated	hr	Uniform (min, max) #
Frequency of contamination	Proportion of cheese with *L. monocytogenes*	%	Triangular (min, mean, max) #
Lag phase	Amount of growth equivalent to lag phase	Log cfu/g equivalent(Subtracted from potential growth to determine outcome)	Lognormal (5,3.87)/3.32 (see section 2.5.1.4)
Coolroom temperature	Temperature during coolroom storage and distribution	°C	Normal (mean, standard deviation)
Coolroom pH	pH as above	-	Normal (mean, standard deviation)
Coolroom salt concentration	Salt concentration calculated from measured water activity	%	Normal (mean, standard deviation)

Min and max values specific to each Case Study

Table 2.2 - Description and distribution of input variables for detailed quantitative risk assessment from consumer handling to consumption

Variable	Description	Unit	Distribution / Model
Consumer temperature	Temperature during retail and consumer handling	°C	Normal (mean, standard deviation)
Consumer pH	pH as above	-	Normal (mean, standard deviation)
Consumer salt concentration	Salt concentration calculated from measured water activity	%	Normal (mean, standard deviation)
Time of consumption	Time during shelf life when cheese is consumed	hr	Triangular (min, mean, max)
Serving size	Amount of cheese consumed in a serving	g	Triangular (5, 30, 125)

Table 2.3 – Derived estimates of input variables for detailed quantitative risk assessment

Variable	Description	Unit	Distribution / Model
Dose	Number of *L. monocytogenes* on serving of cheese	cfu/g of cheese consumed	Serving size x Level at time of consumption
Probability of infection	Probability of listeriosis occurring from dose of *L. monocytogenes* ingested	-	1-EXP(-R* Dose) **Buchanan *et al.* (1997a)** R=1.179*e-10 **Ross (*unpublished*)** R=1.87*e-14
Number of serves	Annual production consumed	-	Annual Production / Serving size

2.4.2 Stochastic modelling sampling methods

In stochastic modelling, two forms of sampling from distributions are commonly used, Monte Carlo (MC) and Latin Hypercube (LH). Vose (1996) considers LH as the better of the two sampling methods as there is "an improvement in reliability and efficiency of Latin Hypercube over Monte Carlo". The randomness of sampling by MC results in a tendency to over- and under- sample from parts of the distribution, and cannot be relied upon to replicate the input distribution's shape unless a very large number of iterations are performed. LH provides a sampling method appearing to be random, but also guaranteeing to reproduce the input distribution with much greater efficiency than MC sampling. The major difference between the two approaches is that LH contains a sampling algorithm that takes into account where the model has previously sampled from within the distribution.

2.4.3 Parameter distributions in the model

To generate useful distribution data, measured process information was pooled at each time interval by following the outlined steps below. Mean values for process temperature, pH and water activity as a function of time were calculated, and upper and lower limits established through addition and subtraction of one standard deviation respectively (results presented in Case Studies). Parameter distributions for temperature, water activity measurements (converted to equivalent salt concentration) and pH were determined by calculating mean values at each time interval. To generate a distribution for these values, data from different times were normalised and pooled as follows. For each time interval, the mean value was subtracted from each measured parameter value, which was then divided by the mean, resulting in a mean value of zero, as shown in Equation 2.1.

Measured temperatures	$t_1, t_2, \ldots\ldots\ldots\ldots t_{10}$	(2.1)
Mean value calculated	$t_m = (t_1 + t_2 + \ldots\ldots\ldots\ldots + t_{10})/10$	
Subtract and divide by mean zero	$(t_1 - t_m) / t_m$ as t_1 approaches t_m, approaches	

Data was sorted and grouped using the histogram function of Microsoft Excel™, and the most appropriate distribution determined using BESTFIT® software (Appendix A). Results for each product are presented in the following Case Studies. The model was created as a spreadsheet model in Excel™ with the @RISK add-in, and these distributions used in the stochastic modelling of the cheesemaking process (see Appendix D for model structure).

2.4.4 Independent variables

A stochastic model must recognise and specifically include any inter-dependencies between parameters. Vose (1996) stated three reasons why a correlation might be observed between data. A logical relationship may exist between two (or more) variables, another external factor may be affecting both variables, or an observed correlation may occur purely by chance and no correlation actually exists. An independent variable is totally unaffected by any other variable within the model. In contrast, a dependent variable is determined in full, or in part, by one or more other model variables. Vose (1996) noted it is extremely important to correctly identify any dependency relationships between variables in spreadsheet stochastic models, otherwise the model may generate nonsensical results.

A scatter plot is a useful method of visualising whether dependency between variables occurs. The common practice is to plot the independent variable on the x-axis and the dependent variable on the y-axis (Vose, 1996). The number of data points generated should produce a scatter plot that fills out low probability areas reasonably well whilst avoiding overpopulation of high probability areas. When deemed appropriate, scatter plots were generated to determine whether interactions existed between process parameters. This process was undertaken and results are presented in each Case Study (Chapters 4-6).

To reflect the correlation between time and temperature during cooling processes, the stochastic model used was created such that, for temperature, pH and salt concentration values selected in an iteration, if the iteration selected a value above the mean, then the value was above the mean in subsequent iterations for the entire process step. The justification for this treatment is that if the product parameter was initially high (or low) compared to the mean, then it would most likely stay high (or low) throughout the process step. This method was used to avoid the model simulating a cooling curve where a temperature is high at one time, low at the next, high at another etc, leading to unrealistic parameter profiles.

2.5 Assumptions necessary for quantitative risk assessment

A number of assumptions are required when assessing the risk of listeriosis from consumption of the specialty cheeses Ricotta, Mascarpone and Brie. As shown in Chapter 1,when conducting risk assessments there are often many elements which are poorly defined. In this case, the usual method has been to make realistic estimates,

based on and extrapolated from existing information. This has been deemed to be a suitable compromise, provided any assumptions are outlined, and any values chosen can be justified to render the risk assessment transparent and open to scrutiny. A number of 'what if' scenarios were also generated to test the robustness of the model, and the assumptions contained within it. This section outlines broader assumptions that were necessary to conduct the detailed quantitative risk assessments presented in the subsequent Case Studies (Chapters 4 – 6).

2.5.1 *L. monocytogenes* Contamination

2.5.1.1 Frequency of contamination

Primarily, the percentage of cheese contaminated will largely determine the extent of the hazard. It is therefore necessary to ensure a realistic estimate of initial contamination rate is made. The quantitative risk assessments conducted in this thesis assumed a level of contamination based on previous factory test results (where available), production statistics and interviews of factory personnel. Previous published surveys detailing prevalence of *L. monocytogenes* in soft cheeses were also examined, where possible, excluding raw milk cheese results (Table 2.4). Pathogen testing conducted during the current study was also included in this data. These results indicated a mean prevalence of 4.3%. As published data does not always distinguish the cheese origin, the survey date taken from European countries may also include raw-milk cheeses. The incidence shown in Table 2.4 was used as the upper limit for contamination frequency, with factory data used to establish the mean value.

2.5.1.2 Time of contamination

In the Process Risk Model presented for each Case Study, the level of *L. monocytogenes* was set to 1 cfu/g at the beginning of each process stage. This allowed the relative contribution of each process step to be evaluated, in terms of predicted *L. monocytogenes* growth.

The assumption was made for the detailed quantitative risk assessment that there was a constant probability of contamination throughout the manufacturing phase. A uniform distribution was used to describe the probability of contamination occurring (Table 2.1). Cheese manufacture, by its very nature, is a very open process allowing the product to be exposed to the air. Contamination may enter the cheese from sources such as aerosols, which can be created within the factory environment very easily during any wash down and cleaning procedures.

Table 2.4 – Data used to estimate prevalence of *L. monocytogenes* contamination in soft cheeses

Ref	Food	Country	Positive	Samples	Prevalence (%)
1	White mould	Denmark, France, Germany, Italy, Sweden	15	154	9.7
2	Soft cheese	Germany	0	42	0.0
3	Soft cheese	Italy	2	121	1.7
4	Soft cheese	Italy	11	222	4.9
5	Soft cheese	Italy	12	90	13.3
6	Soft ripened cheese and Soft unripened cheeses	United Kingdom	67	1135	5.9
7	Brie	United States	0	192	0.0
8	Mould ripened cheese	Various	5	215	2.3
9	Surface ripened cheese	N/A	33	343	9.6
10	Soft / Semisoft cheese	Europe	3	187	1.6
11	Soft cheese	Australia	0	25	0.0
12	Brie, Ricotta, Mascarpone	Australia	0	12	0.0
13	Soft cheese	United Kingdom	72	1130	6.4
14	Soft and fresh cheeses	Brazil	11	103	10.7
15	Soft cheese	United Kingdom	16	1568	1.0
16	Soft cheese	United Kingdom	10	251	4.0
17	Soft cheese	USA	2	100	2.0
18	Soft cheese	Netherlands	4	479	0.8
19	White mould and surface ripened cheeses	N/A	40	604	6.6
20	Mould-ripened cheese	Germany	5	215	2.3
Total			**308**	**7188**	**4.3**

References: 1 Loncarevic *et al.* (1995); 2 Willhelms *et al.* (1991); 3 Massa *et al.* (1990); 4 Pinto & Reali (1996); 5 Tiscione *et al.* (1995); 6 Greenwood *et al.* (1991); 7 Beuchat & Doyle (1995); 8 Pini & Gilbert (1988); 9 Pitt *et al.* (1999); 10 Farber *et al.* (1987); 11 Hobson *et al.* (1991); 12 Miles (unpublished); 13 McLauchlin & Gilbert (1990); 14 Da Silva *et al.* (1998); 15 Gilbert (1995); 16 MacGowan *et al.* (1994); 17 Genigeorgis *et al.* (1991a); 18 Toorop *et al.* (1996); 19 Breer & Schopfer (1988); 20 Terplan *et al.* (1986)

It has been shown that strains of *L. monocytogenes* could survive in aerosols for up to 3.5 hours (Spurlock & Zottola, 1991). It was also assumed that following packaging, no further contamination occurred. This ignored the possibility of cross contamination later in the distribution chain from sources such as retail handling and consumer refrigerators. A uniform distribution was used to select a time at random during the manufacture process, and the Microsoft Excel 'VLOOKUP' function (Appendix A) used to select this time in the process data. The entire manufacture process was included, therefore time of contamination could range from just after heat treatment (contamination immediately after heat treatment) through to packaging (contamination from manual handling of the cheese). The selected time was used as the starting point in the stochastic modelling of *L. monocytogenes* growth in the process.

2.5.1.3 Level of contamination

There was no quantitative data available in this study from the factories manufacturing the cheese products under consideration, as testing for *L. monocytogenes* was conducted on a presence / absence basis. Therefore, level of *L. monocytogenes* contamination was estimated from previous literature reports. Most published data indicating levels of *L. monocytogenes* in cheeses are taken at retail level, therefore prior opportunity exists for the organism to multiply to the reported level. In their extensive survey of UK cheeses, McLauchlin & Gilbert (1990) found an average contamination rate of 404 cfu/g at the retail level. Bemrah *et al.* (1998) estimated a *L. monocytogenes* contamination level of 0 to 33 cfu/mL for raw milk cheeses, while Fenlon *et al.* (1996) showed levels of *L. monocytogenes* on mince at the retail level to be mostly below 20 cfu/g. The requirement for an enrichment step in the standard isolation method, suggests that common levels of environmental contamination are likely to be very low (below the sensitivity of standard spread plate count methodology ie < 10 cell/g). However, the potential also exists for high levels of contamination to occur, as Jeong & Frank (1994) demonstrated that *L. monocytogenes* could reach levels up to 10^6 cfu/cm^2 in biofilms at 10°C on stainless steel.

For the purpose of this risk assessment, the level of *L. monocytogenes* contamination was modelled by a Triangular function (Table 2.1), with parameter estimates based on the literature data outlined above. The minimum possible level of contamination was determined to be a single *L. monocytogenes* cell on a kilogram of cheese, (log –3), the mean value was estimated as 100 *L. monocytogenes* cell on a kg

cheese (log –1), based on and the maximum value 1000 *L. monocytogenes* cfu/g-cheese (log 3), based on upper levels reported by McLauchlin & Gilbert (1990).

The identical contamination rate was used in the model for each cheese, as there is nothing to suggest that one of the cheeses studied is more likely to be contaminated by a larger inoculum of *L. monocytogenes* than any other. The factories were all of good hygienic standard, although only the factory manufacturing the Brie conducted routine environmental sampling for *Listeria* and had extra precautions such as changeover stations for footwear. Such measures were used to alter the estimates of contamination frequency, rather than the contamination level. An increase in numbers due to concentration of the bacteria within the curd (Section 1.5.2.5) was not factored into the model, therefore creating a simplification which must be taken into account when assessing the model outcomes. The assumption was made that all contaminating strains of *L. monocytogenes* were potentially pathogenic, differing from the approach of Bemrah *et al.* (1998) who assumed that a maximum of 10% strains were pathogenic, based on the research of Farber (1996a).

A maximum population density of 10^8cfu/g was assumed, since no growth in excess of this level was observed during challenge test experiments (Chapter 3, Section 3.4) and published results tend to suggest this is the maximum population which occurs at temperatures between 5 and 10°C (Ryser & Marth, 1987b; Genigeorgis *et al.*, 1991; Sulzer *et al.*, 1992; Back *et al.*, 1993). The spreadsheet was constructed so that, once the level of *L. monocytogenes* reached 10^8cfu/g, it remained at this level for the entire time until consumption. No calculation of die-off at stationary phase was incorporated into the model. For purposes of simplification, it was assumed the distribution of contaminating *L. monocytogenes* cells was homogenous throughout the cheese.

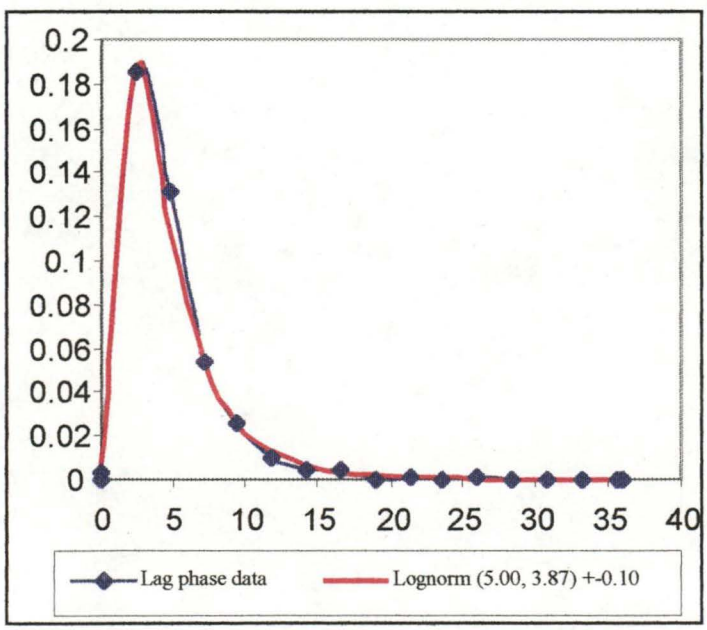
2.5.1.4 Lag time distributions

As stated in Chapter 1, variability in prediction of lag time can be reduced through the concept of relative lag times or the ratio of lag time to generation time (LGR). Ross (1999) demonstrated from published data there was a trend of distribution of relative lag times for a wide range of species across a wide range of conditions. A sharp peak was shown to occur in the range 4 - 6 generation time equivalents, an upper 95th percentile in the range 10 - 15, and a lower 95th percentile in the range 0 - 0 3 generation time equivalents.

The LGR allow lag times observed under one set of growth conditions to be translated into an equivalent lag time under a different set of outgrowth conditions (provided the second set of conditions does not induce significant further lag). This ability can be extended to allow time for resolution of lag time under fluctuating conditions to be calculated. In this situation, the lag time under known conditions is translated into LGR. The potential growth of the population, assuming no lag is then calculated, using an appropriate growth model and the time-environmental history integration technique. The LGR value is then deducted from the cumulative number of generations predicted at the completion of each time interval. No growth is predicted when LGR exceeds the number of generations of growth predicted to have occurred. Once the predicted number of generations exceeds the LGR, the number of potential generations is calculated from the predicted number of generations of growth (from the predictive model) less the LGR.

Literature data detailing 282 lag times for *L. monocytogenes* in response to defined conditions in foods were collated by Ross (1999). This information was entered into Excel spreadsheets and the ratio of lag time: generation time (LGR) calculated. The data were presented as a histogram to determine the mode (most frequently observed value) and spread of LGRs. The data was best described by a Lognormal distribution using BestFit software (Fig 2.1). The fitted equation was used in later stochastic modelling, the outcome divided by 3.32 (the number of generations per log cycle of growth), and subtracting the number of estimated log equivalents spent in lag phase from the total predicted growth.

Figure 2.1 - Comparison of lag phase data with Lognormal distribution



2.5.2 Exposure assessment

2.5.2.1 Time of consumption

Estimates for time of consumption were made based on the shelf life of each individual cheese. Since all products studied here are specialty cheeses, it was assumed that the products are purchased with the intention of consuming the cheeses within a short period of time The assumption was made that a large proportion of the cheese is slightly more likely to be eaten in the first week of the shelf life, but the possibility also exists that the cheese will be consumed after the recommended 'Use-by' date. A Triangular function is used to describe the 'Time of consumption' variable (Table 2.2).

2.5.2.2 Consumption data

The proportion of cheese consumption consisting of Brie, Ricotta and Mascarpone was estimated from ADC production data (Table 2.5). These values were converted into annual consumption values (Table 2.6). However, not all Ricotta, Mascarpone and Brie cheese consumed by the population originates from the factories being studied here. Therefore, it was necessary to estimate the proportion of consumption constituted by the actual brand being studied (i.e. market share). This was possible by estimating the total consumption of the cheese type, and reconciling this against the production figures from factory records. Thus, the final calculated risk of contracting listeriosis was limited to the cheese brands studied here.

Table 2.5 - Australian Cheese consumption (tonnes) 1997-1999

	Total cheese	Ricotta	Mascarpone	Brie
Australian produced	168,888	6,557	3,623	5,936
Imported	33,032	0	0	2,112
Total	201,920	6,557	3,623	8,048
% of Total cheese	100	1.64%	0.90%	2.01%

Adapted from (ADC, 1999)

Table 2.6 - Average daily consumption of cheese by sex and age in Australia

Male age	% who consume cheese	Annual cheese consumption (kg)	Estimated annual Brie consumption (g)	Estimated annual Mascarpone consumption (g)	Estimated annual Ricotta consumption (kg)
2-3	40.2	7.67	153.3	69.0	125.7
4-7	43.1	7.67	153.3	69.0	125.7
8-11	34.4	7.88	157.7	71.0	129.3
12-15	38.7	9.86	197.1	88.7	161.6
16-18	44.8	14.49	289.8	130.4	237.6
19-24	35.8	14.60	292.0	131.4	239.4
25-44	43.8	13.14	262.8	118.3	215.5
45-64	39.2	9.64	192.7	86.7	158.0
> 65	37.9	7.67	153.3	69.0	125.7
Female age	**% who consume cheese**	**Annual cheese consumption (kg)**	**Estimated annual Brie consumption (g)**	**Estimated annual Mascarpone consumption (g)**	**Estimated annual Ricotta consumption (kg)**
2-3	43.6	7.67	153.3	69.0	125.7
4-7	37.0	7.67	153.3	69.0	125.7
8-11	42.2	7.67	153.3	69.0	125.7
12-15	40.5	7.88	157.7	71.0	129.3
16-18	44.5	10.15	202.9	91.3	166.4
19-24	40.3	8.47	169.4	76.2	138.9
25-44	42.9	7.67	153.3	69.0	125.7
45-64	43.0	7.67	153.3	69.0	125.7
> 65	38.7	7.48	149.7	67.3	122.7

Adapted from McLennan & Podger (1999), estimates for Brie, Ricotta and Mascarpone consumption based on ADC (1999)

Australian National Nutrition survey data presented by McLennan & Podger (1999) showed that between 34 and 40% of the population regularly consume cheese. From this, it was assumed that all people who consume cheese also consume the cheese varieties studied here. The same survey presented mean and median values for daily cheese consumption, without distinguishing cheese varieties. When the mean daily values were multiplied to give estimates of total annual consumption figures, they presented a much lower number than that published by the Australian Dairy Corporation (ADC, 1999). However, when the median values from the survey of McLennan & Podger (1999) were calculated to form total annual consumption figures, these were almost identical to ADC figures. Therefore the median values were used to calculate total annual cheese consumption (Table 2.6).

The consumption values listed in Table 2.6 appear low when compared with published estimates from overseas, Bemrah *et al.* (1998) estimated 50 servings per annum of 30g size (annual intake 1.5 kg). Farber *et al.* (1996a) used Canadian statistics which suggested the annual intake of soft cheese was approximately 5.5 kg, with 100g as a typical serving, while Hitchins (1995) estimated the average intake of soft cheese to be 730 g per year.

The estimate of serving size was modelled using a Triangular distribution (Table 2.2), with the minimum value 5g, mean value 30g (based on Bemrah *et al.*, 1998) and the maximum serving size estimated at 125g (based on Farber *et al.,* 1996a). As shown in Table 2.2 the number of serves was based on 2 factors: the serving size (estimated as shown above) and the annual consumption. The number of serves is inversely proportional to the serving size, as the smaller each serving, the more there will be in each 1 kg cheese.

2.5.2.3 Dose response models

The dose response model used in the risk assessment was the exponential model shown in Equation 2.2, as defined by Buchanan *et al.* (1997a).

$$P = 1 - e^{-RN} \qquad (2.2)$$

where:

P is the probability of an adverse effect (Listeriosis)

N is the number of biological agent consumed (cfu), defined as Dose

R is a pathogen specific constant which helps to define the shape of the dose-response curve

Buchanan *et al.* (1997a) estimated a value for $R = 1.179 \times 10^{-10}$ based on the consumption of smoked salmon in Germany, and assuming it is responsible for all observed cases of listeriosis. An estimate of $R = 1.873 \times 10^{-14}$ was proposed by Ross (*unpublished*), based on US data for consumption of a large number of foodstuffs and assuming that reported cases of listeriosis are under-reported by half. Both models were compared to assess which gave the most realistic outcome in terms of listeriosis cases per annum in Tasmania, and in cases where product was exported interstate, the rest of Australia.

2.5.2.4 Susceptible population groups

The consumption data presented in Table 2.6 was calculated for the general population, and does not include population sub-groups which may be more susceptible to listeriosis (Chapter 1). For simplicity, no difference in consumption patterns between the general population and susceptible group was assumed. This may tend to over-estimate the risk, especially as pregnant women have been warned not to consumer these types of cheeses (Health Department of Western Australia, 1995; National Food Authority, 1995). This education campaign may have been effective in reducing soft cheese consumption among some members of the susceptible groups, but this is difficult to evaluate without data to support this assertion. For susceptible groups where consumption data was not available, the mean of the consumption data across the entire population was calculated and used as a default value.

Estimates of the proportion of the general population which is more susceptible to contracting listeriosis range from 15% (Hitchins, 1995) to 20% (Buchanan *et al.*, 1997a). Ross (*pers comm.*) took this one step further by estimating the proportion of the Australian population constituting each of the susceptible groups and comparing with similar estimates made for the Canadian population (Table 2.7). The relative susceptibility of these groups was estimated by Jurado *et al.* (1993) (Table 2.8).

Table 2.9 presents the relative susceptibility for the general population based on listeriosis cases in the US and the proportion that these age groups represent in the Australian population. The most susceptible groups are those under 30 days of age, and those aged over 60 years, comparable with previous observations. This data was combined with Australian Bureau of Statistics data (ABS, 2000) to calculate numbers of people within each population age group and susceptible groups. The generated risk outcome could then be expressed in absolute number of cases per annum, for both the

state of Tasmania, and the rest of Australia. The final risk assessment model, showing all equations and examples of outcomes, is demonstrated in Appendix D

Table 2.7 - Estimates of populations with increased susceptibility to listeriosis

Group	% of total Australian population	% of total Canadian population
Old > 60 years	14.5	12.0
Cancer	0.39	1.00
Transplant patients	0.08	0.04
AIDS	0.11	0.05
Diabetes	1.88	3.9
Pregnant	1.13	1.70
Kidney Disease	0.07	
Total	**18.16**	**17.00**

From Ross (*pers comm.*)

Table 2.8 - Estimates of incidence rates of listeriosis among susceptible populations

Group	Relative susceptibility
Cancer	26
Transplant patients	400
HIV	104
AIDS	230
Pregnant	60

Adapted from Jurado *et al.* (1993)

Table 2.9 - Relative susceptibility to listeriosis based on US incidence data and Australian population data (From Ross, *unpublished.)*

Age	% of cases	% of Australian population	Relative susceptibility
< 30 days	7.4	0.25	29.6
1 – 9 years	1.7	15.3	0.11
10 – 19 years	1.5	15.3	0.10
20 – 29 years	4.4	15.6	0.28
30 – 39 years	12.1	15.4	0.79
40 – 49 years	7.3	13.7	0.53
50 – 59 years	11.7	10.0	1.17
> 60 years	53.9	14.5	3.72

The number of listeriosis cases (per annum) was calculated according to Eqn 2.2. The full model is shown in Appendix D.

(Relative susceptibility) x ((contamination frequency) x (probability of infection per meal) x ((annual consumption) / (serving size))) x ((% of population who consume cheese) x (population of group) x (market share)) (2.2)

This chapter has described the methods, parameter estimates and data which will be used to estimate incidence of listeriosis cases from the cheese products. The justification for parameter estimates must be given, so that the assessment is transparent and open to criticism. In Chapters 4 – 6 each cheese product will be considered through individual Case Studies.

3. PREDICTIVE MODEL EVALUATION

3.1 Introduction

The use of predictive microbiology to model growth of spoilage and pathogenic microorganisms in dairy products has been demonstrated (Langeveld & Cuperus, 1980; Guerzoni, 1994; Griffiths, 1994). However, Rowe (1993) cautioned the value of models may be limited in fermented dairy products such as cheese due to possible antimicrobial activity of starter cultures. The author stated that full validation of models was required before they were used in industry. Most proponents of predictive microbiology have acknowledged that a necessary step in model development is evaluation of its effectiveness in specific food products, before any model can be applied under industrial circumstances. Regardless of how a model performs in fitting a particular data set, the true value of a model lies in how well it predicts microbial behaviour under novel conditions (McMeekin *et al.*, 1993). It must be shown that the model accurately predicts microbial behaviour in the food product under consideration, or at least highlights the model's limitations.

There are two main methods for evaluating model performance. One is through direct comparison of predictions with observed growth rates obtained from challenge tests on the food product of interest. However, because challenge tests are labour intensive and expensive even to gather just a few data points, an alternative approach has been to compare model predictions to the large amount of data that has been published in the scientific literature. A good understanding of the model's ability can then be gathered over a larger range of conditions than could ever be covered in a set of challenge tests.

There are, however, limitations to this technique, as comparison with literature data is often difficult because assumptions have to be made. Authors often do not state the exact conditions under which the experiments were conducted, deficiencies which have been highlighted on numerous occasions (McClure *et al.*, 1994; Zwietering *et al.*, 1994; Ross, 1996; Miles *et al.*, 1997; Neumeyer *et al.*, 1997). Often a_w or pH values are not stated and, therefore, have to be estimated or graphs are presented but no generation time data is calculated. In these cases it is necessary to calculate values manually from an enlarged copy of the graph. The use of literature data can result in more variability, due to the use of different methodology, bacterial strains, inoculum-type etc. However, according to te Giffel & Zwietering (1999) this may encompass a much larger range of real life situations.

To develop confidence for using predictive models in managerial decision making and aid in application of HACCP principles, the accuracy of model predictions during all phases of commercial processing, distribution and storage must be demonstrated. However, van Gerwen & Zwietering (1998) stated it is unlikely one model will adequately cover the entire range of conditions that will be encountered in industry, and in these situations it may be necessary to combine the use of several models to gather suitable outcomes.

3.2 Choice of predictive models

A large number of models to predict *L. monocytogenes* behaviour have been developed, reflecting the level of awareness this pathogen has created over the past 15 years. Models for growth predictions include Buchanan & Phillips (1990); Cole *et al.* (1990); Ross (1993); Wijtzes *et al.* (1993); Guerzoni *et al.* (1994); Houtsma *et al.* (1994); Farber *et al.* (1996b); George *et al.* (1996); Murphy *et al.* (1996); Fernandez *et al.* (1997) and McClure *et al.* (1997). Models are also available to describe survival (Parish & Higgins, 1989), heat resistance (Augustin *et al.*, 1998), and both non-thermal (El-Shenawy & Marth, 1989) and thermal inactivation of the organism (Miles & Mackey, 1994; Chawla *et al.*, 1996; Linton *et al.*, 1996; Buchanan *et al.*, 1997b; Membré *et al.*, 1997) and limits to growth (Tienungoon *et al.*, 2000).

Parameters included in these models are temperature, CO_2, and aerobic/anaerobic atmosphere, as well as the intrinsic parameters water activity, pH, lactic acid, acetic acid and $NaNO_2$ levels. However, none include more than 4 variables, and most were developed from experiments. Laboratory media-derived predictive models are not usually targeted towards specific food products, an approach which was criticised by Murphy *et al.* (1996) and Dalgaard & Jørgensen (1998). Therefore, laboratory-based predictive models rely on the assumption that the modelled parameters are also the controlling factors for microbial growth within the food, and no other inhibitory factors exist. The appropriateness of this assumption can be demonstrated in validation tests.

To maximise the utility of models to everyday situations, a model should contain all parameters relevant to the food product under consideration, and have a wide parameter range. Four models were chosen for evaluation in this thesis.

The Ross-model (Eqn 3.1, Ross, 1993), of the square root model form (Section 1.4.2.2) was developed at the University of Tasmania by Dr Tom Ross, who evaluated the model's performance in several food types and against literature data. It was found that the model performed equally as well as previously published models, and was also applicable for *L. monocytogenes* strains other than the Scott A strain used in developing the model.

$$GT = \frac{1}{\left[(pH - pH_{min}) \times (a_w - a_{w_{min}}) \times (T - T_{min}) \times 0.1971\right]^2} \quad (3.1)$$

where GT = generation time
pH_{min}, a_{wmin}, T_{min} = theoretical minimum values below which no growth is possible (From Ross, 1993)

The Murphy-model (Murphy *et al.*, 1996), utilises simplified natural logarithm (Ln) transformed cubic models for Gompertz parameters M, B and C (Eqns 3.2, 3.3, 3.4). These parameters were used for predicting values for generation time, using the transformation shown Appendix D (Eqns A.1 and A.4). This model was based on the growth of *L. monocytogenes* strain NCTC 5348 grown in sterile reconstituted skim milk powder (10% w/v), developing a model specifically intended for use in dairy products.

$$\text{Ln M} = 43.2892 - 0.4227T - 15.3711*pH + 0.0071*T^2 + 0.0465*NaCl^2 + 2.1299*pH^2 + 0.0286T*pH - 0.0989*pH^3 - 0.00078*T*NaCl*pH + 0.00018*T^2*NaCl - 0.0006*T^2*pH - 0.0028*NaCl^2*pH \quad (3.2)$$

$$\text{Ln B} = -48.0193 + 0.5612*T + 0.1934*NaCl + 18.0587*pH - 0.0098*T^2 - 0.0375*NaCl^2 - 2.6085*pH^2 - 0.0214*T*NaCl - 0.0442*T*pH + 0.1272*pH^3 + 0.0030*T*NaCl*pH + 0.0008*T^2*pH \quad (3.3)$$

$$\text{Ln C} = -29.0536 + 0.0754*T - 0.0674*NaCl + 13.4553*pH - 0.0025*T^2 + 0.0165*NaCl^2 - 1.9810*pH^2 - 0.0032*T*pH + 0.00003*T^3 - 0.0014*NaCl^3 + 0.0969*pH^3 \quad (3.4)$$

where T = temperature, pH = pH and NaCl = sodium chloride concentration (From Murphy *et al.*, 1996)

Models for *L. monocytogenes* growth are also included in the two major predictive modelling databases, the Pathogen Modeling Program Version 5.1 (PMP: Buchanan & Whiting, 1994 - Appendix A) developed by the United States Drug Administration (USDA), and Food Micromodel (FMM: McClure *et al.*, 1994 - Appendix A), developed by the Ministry for Agriculture, Fisheries and Foods (MAFF) in the United Kingdom. These models were evaluated because the computer programs in which they are presented are the most likely to be adopted by industry due to their 'user-friendly' software, as opposed to models taken from scientific literature where a database has to be manually developed. Therefore, it is important to ascertain the appropriateness of these software packages for use in specialty cheese production. The parameter ranges for all models evaluated are shown in Table 3.1. Reported values for each of the model constants were used in all cases, therefore no data fitting occurred.

Table 3.1 - *L. monocytogenes* predictive growth model parameter ranges

Model	Temp (°C)	NaCl (%)	a_W	pH	Atmosphere	Lag model
PMP	5-37	0.5-4.5	-	4.5-7.5	aerobic, anaerobic	yes
FMM	1-35	0-11.5	-	4.4-7.4	aerobic, anaerobic	yes
Murphy-model	3-35	0-8	-	4.5-7.5	aerobic	yes
Ross-model	3-37	-	$\leq$0.997	5.6-7.0	aerobic	no

From Table 3.1 it can be seen all four models contain wide ranges of temperature, a_W (NaCl) and pH. Dalgaard & Jørgensen (1998) previously compared the same four models for prediction of *L. monocytogenes* growth in cold-smoked salmon, and reported that all substantially overestimated growth in naturally contaminated product. On artificially inoculated product, Dalgaard & Jørgensen (1998) found the Ross-model and Murphy-model performed equally well, with the FMM and PMP models performing poorly. When it is considered the intended dairy-specific nature of the Murphy-model, its performance in cold-smoked salmon may tend to suggest the need for models to be food specific is not crucial.

3.3 Materials and Methods

3.3.1 *L. monocytogenes* challenge tests

The primary stage of the model evaluation involved the inoculation of *L. monocytogenes* onto the cheese products being considered for this project. To evaluate model predictions in cheese products, challenge tests were conducted according to the methods outline in Section 2.3. Calculated generation times and lag phases were compared with predictions from the predictive growth models shown in Table 3.1.

3.3.2 Literature evaluation methods

The second stage of model validation compared published reports of *L. monocytogenes* growth rates in similar products. Literature data outlining generation times and lag phases for *L. monocytogenes* in a range of dairy products were gathered from 15 publications, to give a total of 199 generation times and 110 lag phase estimates. A wider range would have been gathered had growth data from laboratory media and other foods been included, but it was deemed more relevant for the purposes of this validation to only include dairy products. The number of data points, model parameter range and any necessary assumptions made with the data sets are listed in Table 3.2.

3.3.3 Bias and Accuracy factors

Evaluation of models was an ill defined process within the field of predictive microbiology, until Bias and Accuracy factors were introduced by Ross (1996), with amendments proposed by Baranyi *et al.* (1999). These factors can be used to evaluate objectively the performance of predictive growth models and provide a guide to the overall performance of the model. The Bias factor (Eqn 3.5) assesses whether the model is 'fail-safe', while the Accuracy factor gives an averaged measure of how close predictions are to observations. A perfect fit is indicated by a value of 1, the more the factor is above this value, the less precise is the average estimate (Ross, 1996). Bias and Accuracy factors can also be used to demonstrate the quality of individual data sets used for comparison.

Table 3.2 - Summary of literature data used for evaluation of models

Ref	observations	Temp range	a_W range	pH range	Assumptions
1	4	4 - 35	0.995	6.4	-
2	3	4 - 15	0.995	6.4	a_W , pH
3	4	5 - 22	0.995	6.5	-
4	10	10	0.995	6.4	a_W, pH
5	13	3 - 10	0.987 - 0.996	6.4 - 7.0	a_W
6	4	7 - 30	0.995	6.5	a_W
7	8	4 - 22	0.962 - 0.995	5.6 - 6.1	-
8	18	5 - 22	0.987 - 0.998	6.31 - 6.50	-
9	4	13 - 35	0.991 - 0.995	5.6 - 6.5	a_W
10	36	12 - 37	0.971 - 0.997	6.6	a_W, pH
11	62	4 - 37	0.986 - 0.995	6.4 - 6.5	-
12	8	13	0.995	6.5	a_W
13	3	6	0.986	6.1	-
14	12	6	0.995	5.6 - 6.8	-
15	10	0 - 9.3	0.997	6.6	-

References: 1 Donnelly & Briggs, (1986); 2 Farber *et al.*, (1990b); 3 Ferguson & Shelef, (1990);4 Marshall & Schmidt, (1988); 5 Murphy *et al.*, (1996); 6 Northolt *et al.*, (1988); 7 Papageorgiou & Marth, (1989b); 8 Papageorgiou *et al.*, (1996), 9 Pearson & Marth, (1990); 10 Rajkowski *et al* , (1994); 11 Rosenow & Marth, (1987a); 12 Rosenow & Marth, (1987b); 13 Ryser & Marth, (1987a); 14 Ryser & Marth, (1988b); 15 Walker *et al.*, (1990)

$$\textbf{Bias factor} \quad 10^{\frac{\Sigma \log\left(GT_{observed}/GT_{predicted}\right)}{n}} \qquad (3.5)$$

where $GT_{observed}$ is observed generation time

$GT_{predicted}$ is predicted generation time

n is the number of observations

A Bias factor less than one indicates the model is 'fail safe', ie observed generation times are larger than predicted values, while a value greater than one indicates the model is 'fail-dangerous'.

$$\textbf{Accuracy factor} \quad 10^{\frac{\Sigma \left|\log\left(GT_{observed}/GT_{predicted}\right)\right|}{n}} \qquad (3.6)$$

The Accuracy factor (Eqn 3.6) averages the distance between each point and the line of equivalence as a measure of how close, on average, predictions are to observations. The larger the value, the less accurate is the average estimate. An Accuracy factor of 2 indicates the prediction is, on average, a factor of 2 different from the observed value (either half as large, or twice as big) (Ross, 1996). The Bias and Accuracy factors can be used to evaluate predictions for generation time and lag phase data.

3.3.4 Visual assessment

Model performance was evaluated by the statistical methods outlined above, as well as graphically. Ross (1996) highlighted the importance of assessing model performance visually. Plotting model predictions against observed values can help to guard against the possibility of systematic deviation in one part of the model being balanced by opposite systematic deviation in another part of the model. This also allows predictions that would be unsafe in practice to be readily visualised. Plots of residuals can also be used to demonstrate if the model is producing biased predictions in any part of the parameter range. Graphical analysis was conducted on the literature validation, as there was not a sufficient number of data points from the challenge tests to warrant graphical analysis.

3.4 Results of Evaluations

3.4.1 *L. monocytogenes* challenge test results

3.4.1.1 Ricotta

Following the inoculation of *L. monocytogenes* onto Ricotta, the cheese samples was stored under both aerobic, and vacuum-packaged conditions (Fig. 3.1). The conditions for growth in the cheese are favourable as it is very moist (a_w =0.997) and the pH value of 6.5 is not a limiting factor. At 20°C *L. monocytogenes* was able to grow rapidly with generation times of 1-2 hr. Predictions from all models were good (Table 3.3), the PMP and FMM models predicting slightly faster generation times, and the Murphy and Ross models predicting slightly slower generation times than those observed. Growth was slowed slightly by the added constraint of vacuum packaging, and the PMP again predicted a faster generation time than was observed. Fig. 3.1 demonstrates how *L. monocytogenes* reached a higher final population under vacuum packed conditions at 5°C, at $\log_{10}$ 6.81 cfu/g, a 10-fold higher population than achieved in aerobically stored cheese where growth levelled out at less than 10^6 cfu/g. Vacuum packing had the opposite effect at 20°C by lowering the final population density, where the growth ceased at less than 10^7 cfu/g, while the *L. monocytogenes* on the aerobically-stored cheese grew above these levels. It is suggested the combination of vacuum packing and lowered temperature (5°C) must allow competition within the product from spoilage bacteria, which may cause *L. monocytogenes* to not reach maximum population density. This situation has been noted previously in meat by Grau and Vanderlinde (1992), and discussed in terms of the Jameson effect, specifically in relation to *L. monocytogenes* (Ross *et al*, 2000).

Under these conditions, the growth rate of the spoilage biota may be such that it outgrows *L. monocytogenes*, causing an inhibitory effect at higher population levels. At 20°C the *L. monocytogenes* appears to be able to outgrow the spoilage bacteria under aerobic conditions and no inhibition is observed.

The PMP and FMM models consistently predicted much faster growth than was observed, whereas the Murphy and Ross models appeared to perform equally well. The growth rates observed here for *L. monocytogenes* in Ricotta under aerobic conditions compare well to those seen in traditional Greek whey cheeses manufactured by a very similar method. At 5°C, Papageorgiou *et al.* (1996) reported

Figure 3.1 - Growth of *L. monocytogenes* on Ricotta (a_W = 0.997; pH = 6.5)

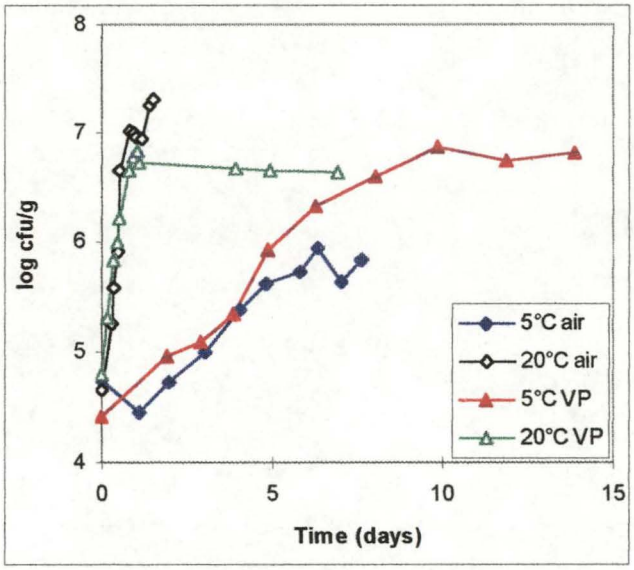


Table 3.3 - Comparison of observed generation times (hr) for *L. monocytogenes* on Ricotta with those predicted by four models

Temperature Atmosphere	Observed	Ross-model	Murphy-model	PMP	FMM
5°C, aerobic	18.2	18.7	17.7	9.5	14
20 C, aerobic	1.3	1.5	1.5	0.9	1.0
5 C, vacuum	20.5	n/a	n/a	7.7	17.3
20 C, vacuum	2.7	n/a	n/a	1.1	1.7

Table 3.4 - Comparison of Observed Lag phase duration (hr) for *L. monocytogenes* on Ricotta with those predicted by three models

Temperature Atmosphere	Observed	Murphy-model	PMP	FMM
5 C, aerobic	52.2	51.1	52.1	54.2
20 C, aerobic	5.5	1.4	6.1	6.1
5 C, vacuum	29.5	n/a	41.8	59.9
20 C, vacuum	none	n/a	4.7	6.0

generation times ranging between 16 and 20 hrs, at 22°C the generation times ranged between 1.7 and 2.7 hrs, comparable to the generation times under similar conditions listed in Table 3.3. Papageorgiou *et al.* (1996) observed maximum populations of *L. monocytogenes* were reached after 24 to 30 days at 5°C, 5 to 12 days at 12°C and 56 to 72 hours at 22°C. Therefore, given the 28 day shelf life of the product, even under refrigerated storage at 5°C, it is still possible for contaminating *L. monocytogenes* to reach very high levels.

Under anaerobic conditions, at 20°C there was no observable lag phase, compared to a lag phase of 5.5 hours under aerobic conditions (Table 3.4). At 5°C with the inclusion of vacuum packing, the lag phase was reduced by around 20 hours. The PMP predicted shorter lag phases under anaerobic conditions, and this was actually the observed situation. Despite this, the predictions were still 'fail-dangerous'.All models gave similarly accurate lag phase predictions for 5°C under aerobic conditions, and the predictions from FMM and PMP were also accurate at 20°C under aerobic conditions. Therefore these lag phase models appear to perform well under aerobic condition, but their performance deteriorates with the introduction of anaerobic conditions.

3.4.1.2 Mascarpone

The average pH (5.9) and a_w (0.992) of Mascarpone allowed growth of *L. monocytogenes*. Growth at 20°C occurred very rapidly (GT ~2-3 hr – Table 3.5), and levels up to 10^8 cfu/g were recorded (Fig. 3.2). Vacuum-packing had the effect of slowing growth and limiting the final density of the organism. PMP gave 'fail-safe' predictions, sometimes by up to 40%. FMM predictions were also 'fail-safe'. The Murphy-model predictions were 'fail-safe', and closer to the observed value. The Ross-model predicted 'fail-dangerous' under both conditions. The lower pH value associated with this product may account for the increasing unreliability of the Ross-model, as it is approaching the lower limit for the model at pH 5.6.

The observed lag phases under vacuum packaging were shorter than those under aerobic conditions (Table 3.6), a similar situation to that observed with the Ricotta. Both the FMM and PMP models predicted a shorter lag phase under anaerobic conditions, but both remained 'fail-dangerous'. None of the models performed accurately for predictions of lag phase in Mascarpone cheese, with predictions up to 10 times longer than observed.

Figure 3.2 - Growth of *L. monocytogenes* on Mascarpone (a_w = 0.992; pH 5.9)

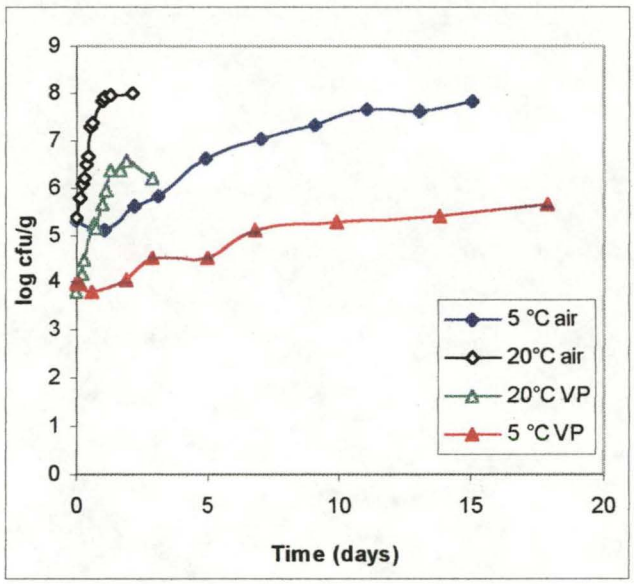


Table 3.5 - Observed and predicted generation times for growth of *L. monocytogenes* on Mascarpone (pH = 5.9, aw = 0.992)

Temperature Atmosphere	Observed	Ross-model	Murphy-model	PMP	FMM
5°C, aerobic	22.4	35.2	19.4	13.7	17
20°C, aerobic	2.0	2.4	1.4	1.3	1.2
5°C, vacuum	44.0	n/a	n/a	9.9	21.5
20°C, vacuum	3.4	n/a	n/a	1.6	2.2

Table 3.6 - Observed and predicted lag phase duration times for growth of *L. monocytogenes* on Mascarpone

Temperature Atmosphere	Observed	Murphy-model	PMP	FMM
5°C, aerobic	29.9	80.3	81.6	121.9
20°C, aerobic	3.0	3.2	9.6	12.2
5°C, vacuum	6.5	n/a	69	74.3
20, vacuum	1.7	n/a	8.1	7.4

3.4.1.3 Brie

The exterior surface of Brie cheese provides a favourable environment for growth of *L. monocytogenes*, with a near neutral pH and a water activity that is not limiting. Conditions inside the cheese are much less favourable with a pH of 5.2. Observed growth curves for *L. monocytogenes* on Brie are presented in Fig. 3.3, demonstrating that at 20°C the organism was able to grow very quickly on either the interior or the surface of the cheese (GT ~ 3 hr – Table 3.7), and capable of growing to very high levels ($\log_{10}$ 8.69 cfu/g) on the surface of the cheese after a period of only 4 days. If the cheese is warmed to room temperature, as is commonly recommended for surface-ripened cheese, and left for several hours, the potential exists for several doublings to take place.

Growth under refrigerated conditions was less pronounced, no detectable growth occurring in the interior of the cheese, and a generation time of greater than one day on the surface of the cheese (Table 3.7). However, since the shelf life of the cheese is greater than 40 days, this is potentially very significant. The maximum population density reached by the organism within the cheese at 5°C was in the order of $\log_{10}$ 6-7 cfu/g, approximately 100-fold less than on the surface.

The Ross-model was limited to predictions for the surface of the cheese because the curd pH was below the range of the model. All models gave predictions at 20°C close to the observed, however, under more extreme conditions the models did not perform as well. None of the models predicted the non-growth situation observed at 5°C in the curd. The Murphy-model gave slightly more accurate predictions than the other models, while the Ross-model gave a 'fail-dangerous' prediction for growth at 5°C on the surface of the cheese. The PMP and FMM models were 'fail-safe' under most conditions.

The results observed here agree with those from several publications which have outlined the behaviour of *L. monocytogenes* on surface-ripened cheeses. Back *et al.* (1993) found significantly higher rates of growth occurred at the surface compared with the centre of Camembert cheese. Ryser & Marth (1987b) suggested pH changes observed on the surface of the cheese, probably due to proteolysis by the white mould ripening process, were obviously a factor in allowing growth of *L. monocytogenes.* Ryser & Marth (1987b) suggested there were no negative interactions between the *Penicillium* mould and the cells of *L. monocytogenes*, a situation that appeared to

Figure 3.3 - Growth of *L. monocytogenes* on Brie surface (a_w = 0.962; pH = 7.02) and interior (a_w = 0.976; pH = 5.2)

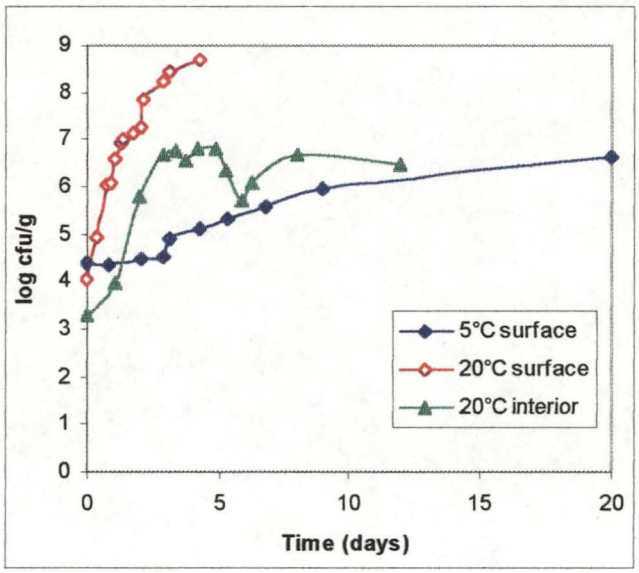


Table 3.7 - Observed and predicted generation times (hr) for growth of *L. monocytogenes* in Brie curd and surface

Temperature	Observed	Ross-model	Murphy-model	PMP	FMM
5°C, curd	no growth	n/a	48.9	39.1	29.3
20°C, curd	3.1	n/a	3.4	3.4	2.2
5°C, surface	25.5	32.4	22.1	17.3	20.7
20°C, surface	2.9	2.2	2.3	1.7	1.5

n/a – out of model range

Table 3.8 - Observed and predicted lag phase duration (hr) for growth of *L. monocytogenes* in Brie curd and surface

Temperature	Observed	Murphy-model	PMP	FMM
5°C, curd	No growth	286.8	355.5	589.6
20°C, curd	18.5	11.3	44.1	47.1
5°C, surface	44	176.3	99.2	226.6
20°C, surface	none	8.2	12.8	15.5

occur in the current study. It has been suggested that higher oxygen levels at the surface of the cheese could stimulate growth of *L. monocytogenes*, with diffusion of oxygen used to explain the results of Back *et al.* (1993), where *L. monocytogenes* grew readily in the centre of cut cheeses.

Lag phase predictions from all models were not very accurate (Table 3.8), on only one occasion was a prediction 'fail-safe', the Murphy-model predicting a shorter than observed lag phase at 20°C within the curd of the cheese. The worst performing model was the FMM which predicted lag phases between three and five times longer than were actually observed. The PMP model also predicted lag phases that were more than twice the observed value.

3.4.2 Literature validation results

Bias and Accuracy factors were used to demonstrate the quality of individual data sets used for comparison (Table 3.9). Bias and Accuracy values for several of the data sets are quite low and tend to suggest some of the assumptions may have been incorrect, or some factor not included in the model has affected growth of *L. monocytogenes*. Data sets consisting of fewer data points tended to behave poorly in the Bias and Accuracy factors, but in the overall evaluation of each model the Bias and Accuracy factors for each individual data point was used, therefore sets with more data did not have a greater effect on overall evaluation of the model.

The Bias and Accuracy values for the literature data evaluation are shown in Table 3.10. As with the challenge test data, it is shown in the literature validation that both the PMP and FMM models generate very conservative predictions, with Bias factors of 0.60 or less. The Accuracy factor shows, on average, the PMP model predicted generation times that were twice as fast as observed. In a recently published literature validation, te Giffel & Zwietering (1999) found Accuracy values for the PMP model on milk, cheese and dairy products to fall between 1.92 and 3.00, and 1.91 and 2.39 for the FMM model, similar to the values observed in the present study. te Giffel & Zwietering (1999) also evaluated the Murphy-model, and calculated Accuracy factors between 1.58 and 2.05 in similar products, worse than the performance calculated here. The current statistical analysis suggests the Murphy-model is the most accurate of the four models compared, with the Ross-model the next best performed.

Further statistical and graphical analysis was conducted to illustrate limitations of the models. To reveal how well the models predict over the entire range of conditions, the literature data were divided into arbitrary groupings of generation times (i) less than 1 hour - near optimal conditions, (ii) between 1 and 5 hours – sub-optimal conditions and (iii) greater than five hours - growth limiting conditions, similar to the approach taken by Ross (1999). Bias and Accuracy factors for each of these data subsets are presented in Table 3.11.

Table 3.9 - Bias and Accuracy factors for individual literature data sets

Ref	PMP		FMM		Ross-model		Murphy-model	
	Bias	Acc	Bias	Acc	Bias	Acc	Bias	Acc
1	0.87	1.19	1.11	1.28	1.35	1.35	1.74	1.74
2	0.36	2.80	0.43	2.31	0.55	2.00	0.53	1.89
3	0.61	1.63	0.72	1.41	1.00	1.24	1.02	1.11
4	0.48	2.08	0.57	1.76	0.61	1.64	0.66	1.50
5	0.51	2.24	0.52	2.33	0.97	1.50	0.83	1.44
6	0.43	2.32	0.50	1.99	0.61	1.63	0.76	1.67
7	0.44	2.34	0.45	2.20	1.67	3.66	0.66	1.81
8	0.50	2.01	0.57	1.75	0.74	1.45	0.74	1.38
9	0.61	1.63	0.65	1.54	1.60	2.45	1.00	1.45
10	0.49	2.04	0.57	1.75	0.68	1.49	0.92	1.16
11	0.45	2.22	0.58	1.73	0.75	1.41	0.78	1.46
12	0.65	1.53	0.72	1.39	0.83	1.26	0.90	1.21
13	0.38	2.60	0.46	2.20	0.67	1.50	0.50	2.00
14	0.62	1.63	0.77	1.41	1.85	2.13	0.95	1.33
15	0.51	1.97	0.74	1.38	2.70	3.48	0.88	1.22

References: 1 Donnelly & Briggs, (1986); 2 Farber *et al*, (1990b); 3 Ferguson & Shelef, (1990);4 Marshall & Schmidt, (1988), 5 Murphy *et al.*, (1996); 6 Northolt *et al.*, (1988), 7 Papageorgiou & Marth, (1989b); 8 Papageorgiou *et al.*, (1996), 9 Pearson & Marth, (1990); 10 Rajkowski *et al.*, (1994); 11 Rosenow & Marth, (1987a), 12 Rosenow & Marth, (1987b); 13 Ryser & Marth, (1987a); 14 Ryser & Marth, (1988b); 15 Walker *et al.*, (1990)

Table 3.10 - Summary of model performance for generation time predictions compared with literature data in foods

Model	Bias	Accuracy
Ross-model	0.88	1.63
Murphy-model	0.82	1.40
PMP	0.49	2.04
FMM	0.60	1.70

Table 3.11 - Performance of models, at optimal, sub-optimal and growth limiting conditions

Generation time	**< 1 hr**		
	n	Bias	Accuracy
Ross	19	0.71	1.63
Murphy	12	1.73	1.73
PMP	19	0.47	2.14
FMM	12	0.69	1.48
Generation time	**1 - 5 hr**		
	n	Bias	Accuracy
Ross	61	0.74	1.57
Murphy	58	0.83	1.32
PMP	61	0.52	1.99
FMM	58	0.56	1.85
Generation time	**>5 hr**		
	n	Bias	Accuracy
Ross	117	0.93	1.55
Murphy	115	0.75	1.42
PMP	111	0.49	2.05
FMM	117	0.61	1.65

Table 3.12 - Summary of model performance for lag phase predictions compared with literature data in foods

Model	Bias	Accuracy
Murphy-model	0.72	2.01
PMP	1.12	1.63
FMM	1.36	1.71

The Murphy-model exhibited the best accuracy with generation times greater than one hour, although these groupings did reveal the model to be 'fail-dangerous' near optimal conditions. The PMP model showed the worst accuracy under all conditions, while the FMM and Ross-models appeared to perform relatively evenly over the entire range. Ross (1999) compared the FMM, PMP and Ross-model for growth of *L. monocytogenes* on meat and flesh-based products, and found all three to have similar accuracy factors. Interestingly, the models predicted 'fail-dangerous' near optimal conditions for the data tested by Ross (1999), not the 'fail safe' values observed with the data from dairy products here.

The comparison of model lag phase predictions with the literature data gave a much better result than observed with challenge test data. The Murphy-model tended to give predictions that were 'fail-safe', although the Accuracy value was greater than two (Table 3.12). The Accuracy values for the PMP and FMM models were very similar, but the Bias factor showed they tended to predict lag phases which were longer than observed, ie 'fail-dangerous'.

3.5 Discussion

3.5.1 Summary of model performance – challenge tests

The use of Bias and Accuracy factors to evaluate model performance objectively demonstrates that both of the commercially available databases (PMP and FMM) considerably underestimate generation times (Table 3.13). These models may have been developed in a deliberately conservative fashion for industry use so they were truly 'fail-safe'. However, as stated by Dalgaard & Jørgensen (1998), models which give predictions which are too 'fail-safe', indicating pathogen growth to be much faster than actually observed in naturally contaminated product, can result in unrealistic or awkward measures to be used in risk management. The Bias factor of

0.92 demonstrates the Murphy-model provides 'fail-safe' predictions which are within 20% (Accuracy factor = 1.20) of the observed value. The Ross-model also performed quite well, giving the second closest predictions (Accuracy = 1.27), however the Bias factor of 1.19 shows the predictions tend to be on the 'fail-dangerous' side.

Table 3.13 - Summary of model performance for challenge test generation time data

Model	Bias	Accuracy
Ross-model	1.19	1.27
Murphy-model	0.92	1.20
PMP	0.57	1.78
FMM	0.68	1.46

All models performed badly with predictions of lag phase, with the PMP and FMM model predicting lags on average 3 times longer than were observed. The Murphy-model performed best, however it still gave 'fail-dangerous' predictions which were, on average, almost twice as long as observed. It also must be noted the Ross and Murphy-models were not used for anaerobic conditions (a limitation of the models), which may have increased its accuracy compared to the other two models. However, when Murphy-model predictions were extrapolated to anaerobic conditions, the prediction were often more accurate than the dedicated anaerobic models of both the PMP or FMM models.

Table 3.14 - Summary of model performance for challenge test lag phase data

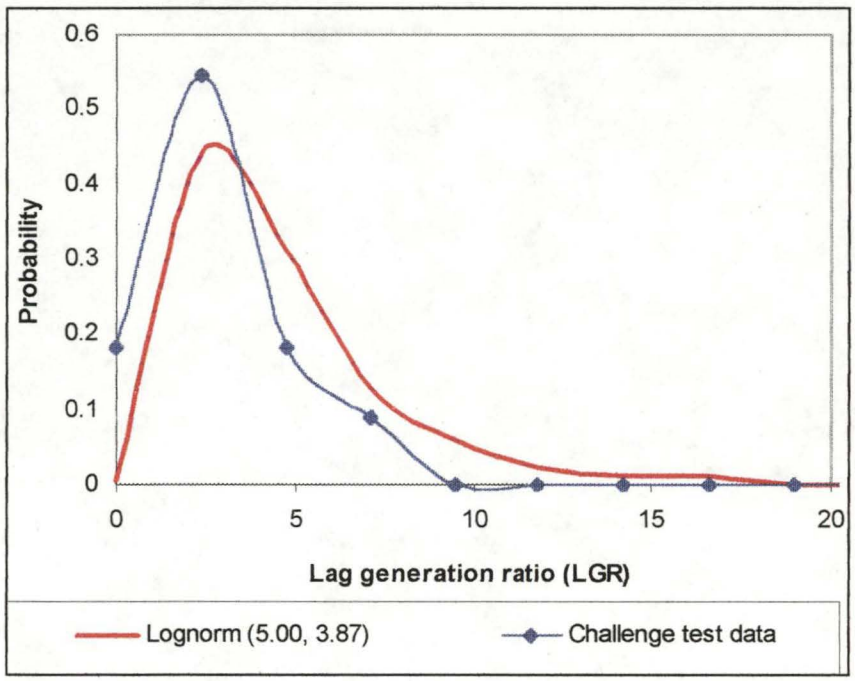
Model	Bias	Accuracy
Murphy-model	1.68	1.91
PMP	3.13	3.13
FMM	3.46	3.60

The Bias and Accuracy values from the challenge tests suggest the reliability of these three models for predicting lag phases in Ricotta, Mascarpone and Brie is not good. At optimal temperatures it may be safest to assume no lag phase, as was observed in some of the current experiments. The inoculum used in these experiments contained high levels of exponential phase cells, therefore these challenge tests imitated the "worst case scenario". The influence of inoculum size on lag time variability has been discussed by several authors (Baranyi & Pini 1999; Augustin *et al*, 2000), with lower inoculum levels leading to increased lag time variability. Naturally contaminated product may be due to cells which are injured, therefore observed lag phases may be longer, and ultimately closer to the predicted lag times. The addition of vacuum-packaging to the product may extend the shelf life through inhibition of spoilage bacteria, but there appears to be mounting evidence *L. monocytogenes* does not grow much more slowly under anaerobic conditions, and lag phases can even be shorter under anaerobic conditions (Back *et al.*, 1993). These findings were supported by the observed growth in Ricotta and Mascarpone.

It was shown in Section 2.5.1.4 that lag time may be best expressed in terms of a lag time : generation time ratio (LGR), given the variability of lag time responses and the inherent inaccuracy in attempting to model it. Several authors (Adair *et al.*, 1989; Zwietering *et al.*, 1991; Baranyi & Roberts, 1994) have observed the lag time response to environmental conditions is often proportionally the same as the generation time response, i.e. conditions which double the generation time will also double the lag time or conditions which reduce generation time four fold will also reduce the lag time four fold. This relationship could be used to estimate lag phase duration based on growth rate predictions.

A comparison of the LGR was undertaken with the challenge test data generated in this study. The distribution which was presented in Fig 2.1 demonstrated that lag phase was best described by a Lognormal distribution, the fitted parameter of which were selected by Bestfit software (Lognormal 5.00, 3.87). The suitability of this distribution for modelling *L. monocytogenes* lag phases was tested by comparing it against the observed lag phases calculated from the challenge tests conducted on the Ricotta, Brie and Mascarpone. Given that there were only 11 data points from the challenge tests, it can be seen that the Lognormal distribution described in Chapter 2 appears to fit the data well.

Figure 3.4 – Comparison of challenge test Lag time : Generation time ratio data and Lognormal distribution selected by BestFit software (from Chapter 2)



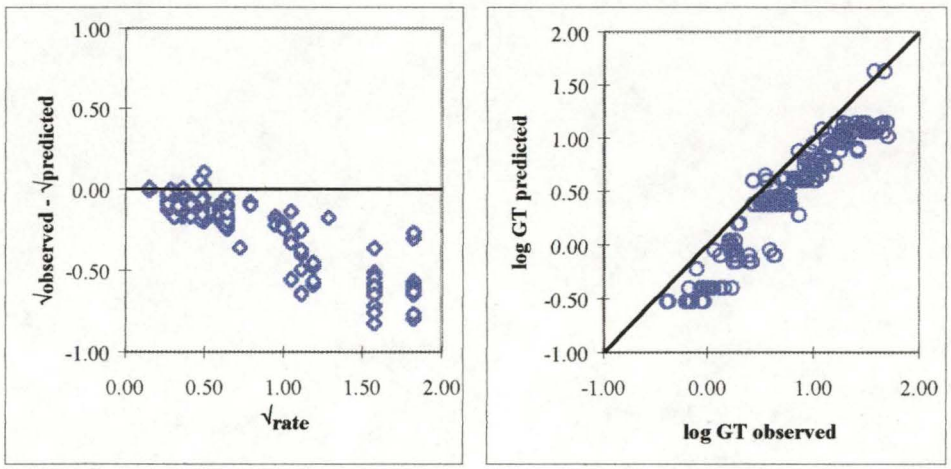
3.5.2 Literature validation

3.5.2.1 Pathogen Modeling Program (PMP)

For both challenge test data and literature data, the PMP was the worst-performing model, predicting generation times that were much faster than were observed. The model is 'fail-safe', but it is so conservative with its predictions as to be of little use in the food industry. The literature validation Bias factor was 0.49 and Accuracy was 2.04, indicating the model is predicting generation times, on average, twice as quick as those observed, while the Bias factor for the cheese challenge tests was marginally better at 0.63. The results of the validation with literature are shown in Fig. 3.5, Fig. 3.5a shows most points tend to fall well below the line of equivalence, thus the model is systematically under-predicting generation times. Fig 3.5b also shows that, under more favourable conditions (ie shorter generation times), the model tends to under-predict even more, with the scatter of residuals heading further towards negative values.

Figure 3.5 - Comparison of literature data with predictions from Pathogen Modeling Program

3.5a log-log plot **3.5b residual plot**



Dalgaard & Jørgensen (1998) found a similar situation where the PMP could not be successfully validated in smoked salmon because it predicted growth rates which were too fast. Papageorgiou *et al.* (1996) on whey cheeses also found predictions from PMP to be faster than observed. Rowe (1993) stated the PMP model consistently underestimated the generation time and overestimated the lag phase duration for growth in whole milk and 2% milk. However, the author did comment the model is particularly useful because it lends itself to the development of user-friendly software.

Alternatively, the lag phase predictions from PMP are 'fail-dangerous', predicting lag phases which are, on average, three times as long as observed. This is in agreement with the results of Walls & Scott (1997a), who showed lag phase predictions by PMP to be 'fail-dangerous' on most occasions with inoculation of *L. monocytogenes* onto sterile baby food. Little & Knøchel (1994) evaluated the PMP models for *Salmonella*, *Bacillus* and *Yersinia enterocolitica*, comparing predictions with observed growth rates on Brie. They found in some cases the predicted lag phase was longer than observed, ie 'fail-dangerous'. Little & Knøchel (1994) attributed the failure of the models to produce accurate predictions in some cases was due to the fact the models were constructed from growth data obtained in culture broth media, free from natural inhibitors and competing microorganisms. It was hypothesised by the authors that high numbers of lactic acid bacteria present in the cheese may reduce the growth of these foodborne pathogens. This did not appear to be the case with *L. monocytogenes* in

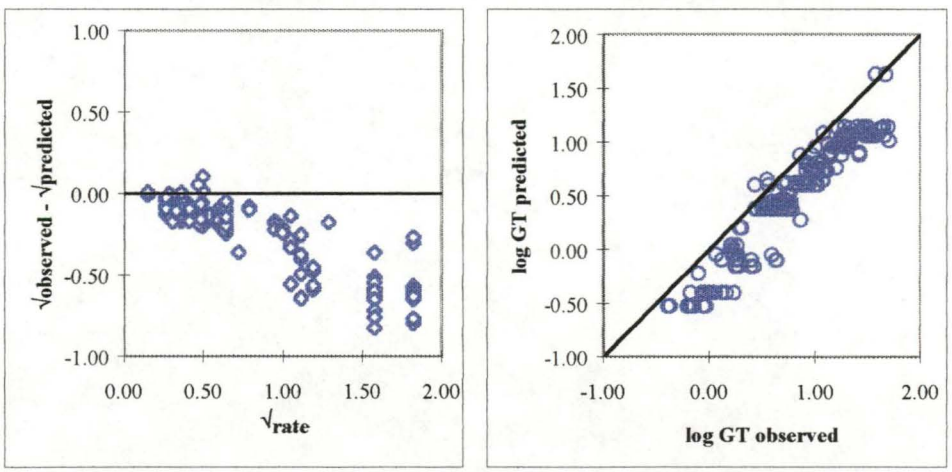
any of the cheeses studied here. This evaluation of the PMP *L. monocytogenes* model has revealed some marked deficiencies in the model. Although the model predicts 'fail-safe', the conservative nature of the model renders it unsuitable for the purposes of the work being undertaken here, and for the dairy industry in general.

3.5.2.2 Food Micromodel (FMM)

The FMM model performed marginally better then the PMP, but was still the second worst of the four models evaluated. A Bias factor of 0.70 was achieved in challenge tests, and 0.60 in the literature validation. Again, the predictions were very conservative and tended to be too fast when compared to observed growth rates. The FMM model also appears unsuitable for predictions in the cheese products being studied here. Fig. 3.6a shows a similar pattern as seen in the graphical analysis of the PMP model, with the majority of points falling below the line of equivalence. Fig. 3.6b also shows a similar trend to the PMP model, with residuals tending to become more negative at higher growth rates.

Figure 3.6 - Comparison of literature data with predictions from Food Micromodel

3.6a log-log plot **3.6b residual plot**



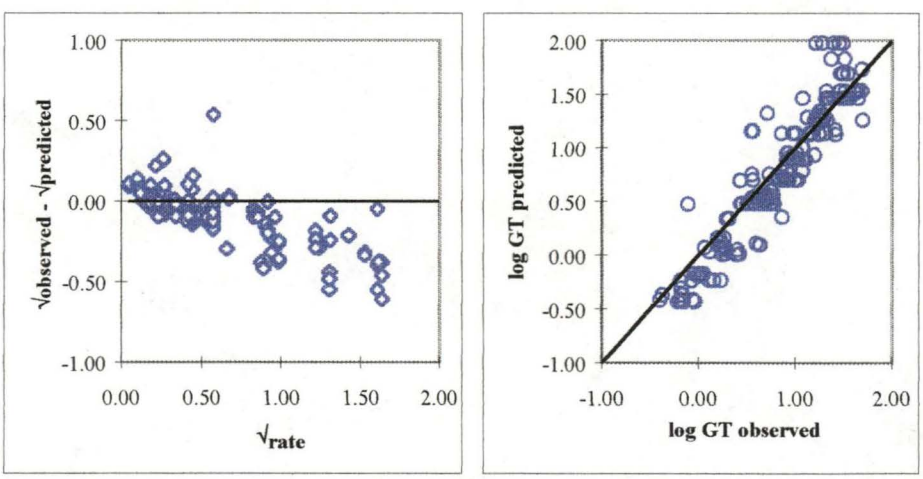
The lag phase component of the model fared little better in the evaluation, it was the worst performed in the challenge tests with a Bias of 3.46, and 1.36 for the literature data. Walls & Scott (1997a) also showed the lag phase model from the FMM generally was 'fail-dangerous'

3.5.2.3 Ross-model

The Ross-model was the simplest model evaluated, and it provided the second best predictions. In the challenge tests the Bias factor was 1.19, indicating the model tended to be slightly 'fail-dangerous', and it was the second closest to the actual observed generation times, the Accuracy factor was 1.27 indicating the prediction were within 27% of observed. The amount of challenge test data used to evaluate the Ross-model was slightly less than for the FMM and PMP models, as the former did not include estimates for anaerobic atmosphere. With less data in the sub-optimal growth region with which to evaluate the Ross-model, this may have helped its performance when compared to the other models. In the literature validation, the Ross-model performed very well with a Bias of 0.88 (1st) and Accuracy of 1.63 (2nd). Fig. 3.7a shows the scatter of points around the line of equivalence, while Fig. 3.7b shows a similar trend to those observed for the FMM and PMP models, with residuals becoming more negative at higher growth rates.

Figure 3.7 - Comparison of literature data with predictions from Ross-model

3.7a residual plot **3.7b log-log plot**



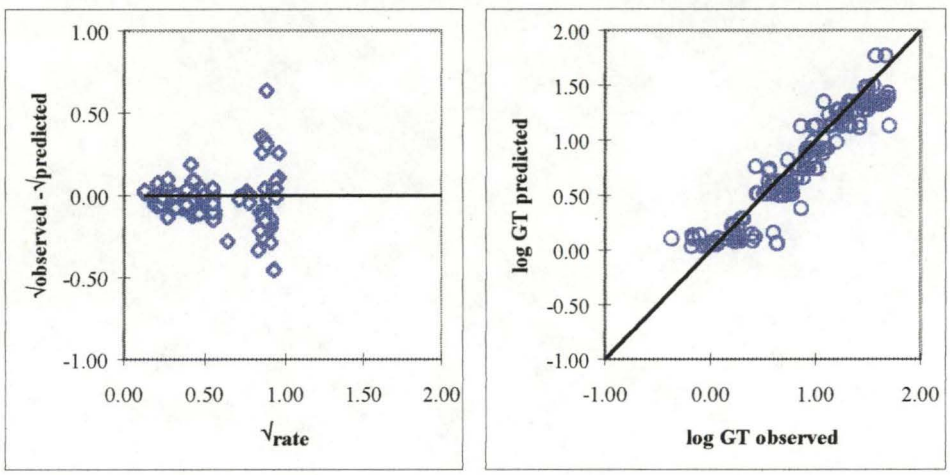
3.5.2.4 Murphy-model

The Murphy-model was the best of the models tested, giving 'fail-safe' predictions for both the challenge test data (Bias 0.92) and literature data (0.82). The Accuracy factors were the best for both sets of data, suggesting this model gives the most realistic predictions of generation time. The scatter of points around the line of equivalence, with no obvious signs of under- or over-prediction is shown in Fig. 3.8a. The same Bias in residuals observed for the other three

models was not displayed by the Murphy-model. Residuals were scattered around zero, although the scatter becomes more pronounced at higher growth rates (Fig. 3.8b).

Figure 3.8 - Comparison of literature data with predictions from Murphy-model

3.8a residual plot **3.8b log-log plot**



The performance of the lag phase model was not as good, with a Bias factor of 1.68 on the challenge tests, and 0.72 on the literature data. Despite this, it was still the best performer of the three models used for predicting lag phases.

The limitation of this model for application to the cheese products under consideration here is that it does not include a term for atmosphere, thereby limiting its effectiveness as far as vacuum packed product. The application of vacuum packaging appeared to slow growth of *L. monocytogenes* slightly, therefore it may be justified to use the Murphy-model in these instances because the model will still be 'fail-safe'. Despite not containing a model for anaerobic growth, the performance of the Murphy-model under these conditions was better than either of the other two models with specialist anaerobic models.

3.5.3.5 Lag phase modelled by Lag : generation ratio (LGR)

It was shown in Section 3.5.1 that the Lognormal distribution was able to describe the calculated LGR values for the challenge test data. An evaluation of the LGR was unable to be undertaken for the literature section of the validation study. The published lag phase data did not have the accompanying generation

times was considered to be of little value. An evaluation of this kind would be affected by the accuracy of the generation time model, and thus the resultant distribution would be of little practical significance. Given the demonstrated limitations, through the Bias and Accuracy factors, of the lag phase models, and the demonstrated ability of the Lognormal distribution to accurately describe the duration of *L. monocytogenes* lag phases (Ross, 1999), this Lognorm distribution was incorporated into Stochastic modelling of *L. monocytogenes* growth (as shown in Appendix D).

3.5.3 Conclusion

Most research on pathogen survival during cheesemaking has utilised laboratory-cultured milk inoculated with fairly high numbers cultured under favourable conditions for cell proliferation. The pathogens in naturally infected milk which survive heat-treatment and enter the cheesemaking process are probably relatively few in number and not in the same physiological state as inoculum cultured under favourable conditions. The survival of pathogens in the sequential heat-treatment - cheesemaking processes should be researched, utilising "naturally" contaminated milk where feasible.

Model accuracy has been generally shown to decrease as the degree of experimental control is reduced. Use of non-sterile, non-homogenous food or literature data results in lower levels of confidence. Therefore one approach has been to use sterile, homogenous baby food to validate models (Walls & Scott, 1997a). While this may give a higher level of experimental control, it is doubtful whether it reflects reality, i.e. the everyday conditions which the model will be expected to make valid predictions under. To evaluate a model properly, comparisons with growth rates in the product of interest need to be carried out. Literature data can be used as an aid in evaluating a model, because the volume of data that can be included is much greater. Considering the assumptions made with the literature data the overall performance of the Ross and Murphy models appear to be quite good.

The use of sterile food may increase the accuracy of a model, but to evaluate predictions in a cheese, the actual product complete with starter and spoilage bacteria needs to be used. If these outside influences affect the accuracy of a model, then this must be considered when making management decisions based on the model's output. The results presented in this chapter demonstrate that models are of value to predict the growth of *L. monocytogenes* in cheese products. The model developed by Murphy

et al. (1996) provided a suitable model for growth predictions in the cheese products considered here, and also fitted the literature data for a broader range of dairy products. The Murphy-model gave the best fit to the data of all the models considered here and will be used in all subsequent modelling of *L. monocytogenes* growth during cheesemaking process, storage and distribution. Ideally an integrated growth / survival / death model could be used to evaluate the whole continual food process. Otherwise it is a matter of comparing models to determine which gives the most appropriate predictions to the product in question. The generation time predictions from the models were generally quite good, however the ability to model lag phases accurately remains a problem in predictive microbiology.

A suitable model has now been identified. The next step will be to consider each specialty cheese product in turn, identifying which process steps contribute most to the risk of *L. monocytogenes* contamination and growth, as an objective means of identifying Critical Control Points.

4. PRODUCT CASE STUDY 1 – 1 KG BRIE

4.1 Introduction

This Case Study presents a quantitative risk assessment on the consumption of 1 kg Brie manufactured by Factory 'A'. The cheesemaking process is modelled using the Murphy *et al.* (1996) model for *L. monocytogenes* growth prediction. The predicted level of *L. monocytogenes* growth in the cheese is converted, through an exposure assessment, into an estimated number of listeriosis cases due to consumption of the cheese in a year.

4.1.1. Brie

Brie is soft creamy cheese enclosed in a white surface rind of mould and is traditionally made in the shape of a large flat disc. It is one of the most popular cheese varieties, not only in France where it was first developed, but all over the world. Brie takes its name from a province in the Marne valley to the east of Paris (Davis, 1976). The origins can be traced to farms in the Ile-de-France around Paris, although it is now made in many parts of the world. References to Brie appear in many French historical documents and it is recognised as the original surface-ripened cheese (Rogers, 1995). Brie shares many of the characteristics of Camembert, with most soft cheeses produced by similar technology, made from a soft curd (pâte molle) and not cooked, pressed or worked in any way, and only lightly salted (Davis, 1976). All varieties of Brie are made in virtually the same way, differing only in size, treatment of milk (fat content) and degree of ripeness when consumed. The white mould used on the surface of the cheese is *Penicillium camemberti*, which results in a relatively high moisture cheese, along with a neutral pH in fully ripened cheese. The Australian market for surface-ripened cheese has not grown significantly over the last eight years, with a slight decrease in three of the last four years. However, Australian-produced cheese has increased its market share from 30% to 70% replacing imports (Willman, 1998), allowing significant increases in the Australian production of surface-ripened cheeses.

It is recommended to warm Brie prior to serving, to enhance the aromatic properties of the cheese (Rogers, 1995). Therefore, it is common practice for many consumers to remove the cheese from the refrigerator several hours before serving, subjecting it to a mild temperature abuse. The cheese is often used in tortes for desserts, on cheese boards and may also be used for deep-frying. In some cases, Brie can be packaged in a tin and heat-treated to extend the shelf life. The resulting

product is uniform in flavour and texture and does not require refrigeration until opened. Other varieties of Brie include double cream, triple cream, Brie de Meaux, Brie de Coulommiers, Brie de Melun, and Brie flavoured with peppers, herbs, mushrooms or sundried tomatoes. Factories within the state of Tasmania manufacture several of these varieties. The hazards inherent in these products may differ from the cheese considered in the present study due to post-pasteurisation inclusions and higher moisture content.

Several published studies have shown that surface ripened cheeses such as Brie can support the growth of pathogenic organisms. Little & Knøchel (1994) demonstrated that growth of the pychrotrophic pathogen *Yersinia enterocolitica* could occur over the temperature range 4 to 20°C on Brie, while *Salmonella* and *Bacillus cereus* grew only at 20°C. Genigeorgis *et al.* (1991b) showed that both Brie and Camembert could support the growth of *L. monocytogenes* over the temperature range 4 to 30°C. This was assumed to be due to the high pH at the surface (7.4) and the centre (6.9) of the samples. Both of these studies highlighted that the surface mould did not appear to inhibit pathogen growth. Starter culture bacteria, present at levels of up to 10^8cfu/g also did not appear to have any adverse effect on growth of *L. monocytogenes* (Genigeorgis *et al.* 1991b). Growth was found to be more rapid on the surface than in the interior of the cheese. One of the main factors enabling surface ripened cheeses to support the growth of *L. monocytogenes* is thought to be the increase in pH associated with the white mould ripening process and the resultant proteolytic release of ammonia (Ryser & Marth, 1987b). During the latter stages of maturation, when the internal pH of the cheese begins to rise, those authors observed rapid growth in the centre of the cheese at temperatures commonly used for maturation.

4.2 Methods and Materials

4.2.1 Characterisation of Brie cheesemaking process

The cheesemaking process was characterised according to the method outlined in Section 2.2, commencing at the point of milk leaving the heat exchanger and entering the fill tank. Parameter distributions were established for temperature, pH and salt concentration (calculated from a_w) by the transformation shown in Section 2.4.3. The Brie manufacture process was divided into separate stages to allow improved process parameter definition. These were arbitrarily defined as Production (0-24 hours), Draining (24-48 hr), Maturation (days 3-9), Storage and

Transport (day 10-13) and Shelf Life (day 14-59). Due to rapid fluctuation in parameter values during the initial cheesemaking process, the production stage was further subdivided into 4 hour segments. The calculated parameter distributions were used in subsequent stochastic modelling of the process.

4.2.2 Analysis of Brie final product attributes

Final product samples were analysed 24 hours after wrapping to characterise the distribution of pH and calculated salt concentration (from measured a_w values). Standard plate count, lactic acid bacteria, yeast and mould counts were evaluated according to the methods outlined previously in Chapter 2 and the mean values, standard deviations, maxima and minima determined.

After characterisation of the cheesemaking process and finished product, a 'rough' or Process Risk Model was conducted following the modelling strategy outlined in Section 2.4.1. A second, more detailed risk assessment model was subsequently established to estimate the risk of listeriosis. As stated in Chapter 2, there is a scarcity of data for aspects of risk assessment, requiring assumptions to be made. The necessary assumptions, specific to this Case Study are listed in Section 4.2.3 below.

4.2.3 Risk assessment - assumptions

In addition to the assumptions outlined in Chapter 2, several additional assumptions were necessary to conduct a detailed quantitative assessment of risk of contracting listeriosis from the consumption of Factory 'A' 1 kg Brie. Inputs where specific values were inserted for this Case Study are shown in Table 4.1.

Table 4.1 – Distribution inputs for Detailed Brie risk assessment

Variable	Description	Unit	Distribution / Model
Frequency of contamination	Proportion of cheese contaminated with *L. monocytogenes*	%	Triangular (0, 0.003, 0.043)
Time of contamination	Time during manufacture when cheese is contaminated	hr	Uniform (0, 216)
Time of consumption	Time during shelf life when cheese is consumed	hr	Triangular (314, 500, 1416)

4.2.3.1 Frequency of contamination

The frequency of *L. monocytogenes* contamination was estimated from positive isolations from Factory 'A's product over the last three years. During that time the factory had one batch of cheese contaminated with *L. monocytogenes*. The volume of affected product was calculated as a proportion of the entire production during the last three years, leading to a mean frequency contamination value of 0.3%. The upper limit for contamination was estimated using data from Table 2.3. A Triangular distribution was estimated to establish lower and upper limits of 0 and 4.3% contamination respectively (Table 4.1).

L. monocytogenes was not detected in any of the Factory 'A' Brie samples (n = 10) tested in the current study. A system of GMP was in place within the Factory, with a footwear exchange to limit traffic through the manufacturing room and prevent outside contaminants being introduced into the factory. Extensive factory and environmental *Listeria* surveillance was an integral part of the Food Safety Schemes developed by the factory (Table 4.4). Therefore the use of 4.3% contamination as the upper limit may be an overestimation of the actual situation, but reflective of what may occur should undetected contamination take place. Simulations were also conducted assuming a much lower frequency (0, 0, 0.3%), to gauge the effect on the number of listeriosis cases.

4.2.3.2 Time of contamination

A microbiological profile of the process was conducted to detect potential contamination sources. The results (Section 4.3.31) justified the selection of a uniform distribution for the time of contamination (Table 4.1).

4.2.3.3 Time of consumption

The shelf life specified on the product label by the manufacturer is expressed as a 'Best-before' date. This falls between 45-52 days from day of manufacture, as the entire week's production has the same 'Best-before' date. Therefore the nominal shelf life is dependent on the day of the week the cheese was manufactured. The labelling includes recommendations for storage by the consumer, reading "*Please keep refrigerated between 2°C and 5°C*". However, the label can also be used as a guide to how the cheese may be handled by the consumer. It states that the Best-before date "*..indicates when the cheese is at its peak condition. It is still delicious*

before that date, but its texture and flavour will be slightly different. Depending on your personal taste, this cheese can also be enjoyed beyond the best before date".

For the stochastic model, a worst-case scenario of the full 52 day shelf life was assumed, with an extra 7 days included to account for consumers who may eat the cheese after the suggested 'Best-before' date. In the current study, the cheese was found to be visually and organoleptically acceptable at the conclusion of the shelf life. Therefore it was considered not to be unusual for the cheese to be consumed after the 'Best-Before' date. The shelf life of the product was profiled at two temperatures, 5°C and 10°C to simulate typical storage temperatures and mild temperature abuse respectively. The microbiological profile (Total plate count, lactic acid bacteria, yeasts and mould) and intrinsic parameters (pH and a_w) of the product were monitored during the shelf life by the methods outlined in Section 2.2.3.

4.2.3.4 Exposure assessment

Point estimates of consumption data for each population group (as shown in Chapter 2) were entered into the model. The total amount of cheese consumed was calculated by multiplying the proportion of the population who consume cheese, by the average annual consumption. These values were summed and compared to factory records of annual production of Brie at Factory 'A', to determine what proportion of market share that Factory 'A' cheese must constitute. This was to ensure that calculated consumption values did not exceed production volumes. Approximately 125 tonnes of Factory 'A' Brie cheese is manufactured *per annum*, with approximately 25 tonnes sold within the state of Tasmania (an estimated market share of 70%), and 100 tonnes exported interstate to the rest of Australia (an estimated market share of 7%).

4.3 Results

4.3.1 Characterisation of Brie manufacture process

The manufacture of 1 kg Brie in Factory 'A' is a 9-day process, with cheese packaged on the morning of the tenth day. A typical manufacturing schedule is shown in Table 4.2. Temperature, pH and calculated salt concentration profiles are shown in Figs 4.1a, 4.1b and 4.1c. Milk is initially stored at 4°C in bulk tanks before undergoing a sub-pasteurisation heat process (thermalisation) and pre-

ripening. The milk is cooled overnight and then subjected to a full pasteurisation heat treatment at 73°C for 15 seconds.

Table 4.2 - Typical manufacturing schedule and parameter values for 1kg Brie

Step	Time		Temp (°C)	pH	a_w
Tank fill	Day 1	7:30 AM	39.2 ± 0.4°C	6.45 ± 0.11	0.996 ± 0.001
Add starter culture		7:55 AM	38.9 ± 0.6°C	6.41 ± 0.04	0.996 ± 0.001
Add mould		8:00 AM	38.7 ± 0.8°C	6.40 ± 0.05	0.995 ± 0.001
Bassine fill/add rennet		8:40 AM	38.2 ± 1.2°C	6.38 ± 0.05	0.995 ± 0.001
Cut curd		8:55 AM	37.8 ± 1.2°C	6.35 ± 0.07	0.995 ± 0.001
Stir curd		9:05 AM	37.9 ± 1.3°C	6.33 ± 0.09	0.995 ± 0.001
Hoop		9:25 AM	37.3 ± 1.3°C	6.29 ± 0.12	0.994 ± 0.002
Turn 1		11:25 AM	32.9 ± 1.8°C	5.67 ± 0.16	0.993 ± 0.002
Turn 2		1:25 PM	30.0 ± 1.7°C	5.32 ± 0.02	0.993 ± 0.001
Dehoop	Day 2	4:30 AM	15.3 ± 1.2°C	5.04 ± 0.06	0.980 ± 0.004
Brine		5:00 AM	14.3 ± 1.3°C	5.04 ± 0.07	0.975 ± 0.007
Drain		6:30 AM	11.6 ± 0.5°C	5.02 ± 0.09	0.964 ± 0.011
Spray mould		10:00 PM	12.6 ± 0.6°C	5.10 ± 0.01	0.959 ± 0.008
Move to maturing room	Day 3	6:30 AM	12.4 ± 0.6°C	5.14 ± 0.05	0.965 ± 0.004
Turn 1	Day 5	4:00 PM	12.7 ± 0.9°C	5.14 ± 0.03	0.967 ± 0.004
Turn 2	Day 7	4:00 PM	13.1 ± 0.9°C	5.43 ± 0.22	0.968 ± 0.002
Move to coolroom	Day 9	5:00 AM	11.7 ± 2.5°C	6.96 ± 0.24	0.965 ± 0.001
Wrapping		7:00 AM	10.8 ± 2.7°C	7.03 ± 0.22	0.965 ± 0.001

Figure 4.1a – Average temperature profile of Brie cheesemaking process (—) with upper and lower limits (—)

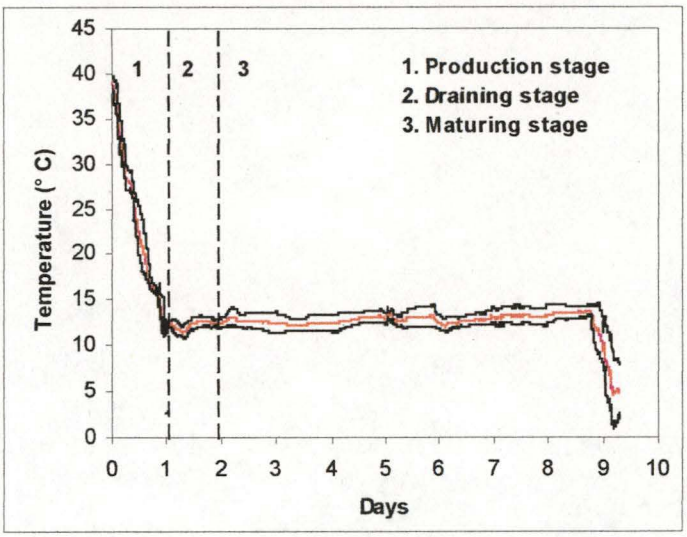


Figure 4.1b – Average pH profile of the Brie cheesemaking process (—) with upper and lower limits (—)

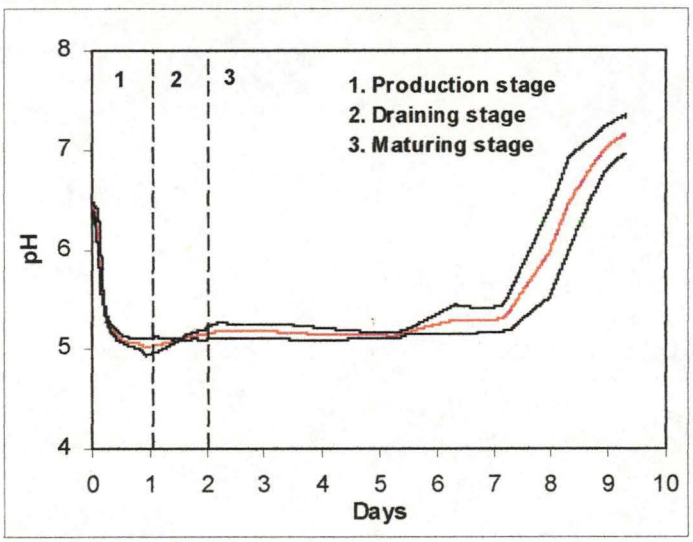
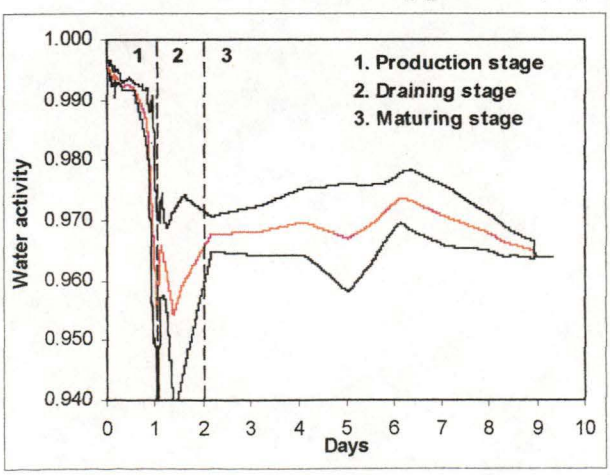
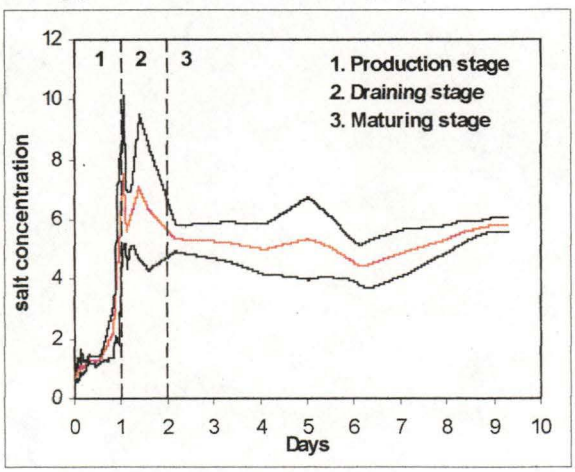


Figure 4.1c – Average water activity profile and equivalent salt concentration of the Brie cheesemaking process (—) with upper and lower limits (—)





4.3.1.1 Brie Ingredients

Ingredients used for Brie manufacture are milk, salt, calcium chloride, Lysactone (Glucono Delta Lactose), skim milk powder, rennet, starter cultures and mould. The ingredients that are most likely to be contaminated and thus constitute the greatest risk are the skim milk powder and raw milk. Skim milk powder is added prior to pasteurisation to standardise fat content, and raw milk is pasteurised at 73°C for 15 seconds, in excess of the minimum requirements (ANZFA, 1999). It was shown in Chapter 2 that normal High Temperature Short Time pasteurisation is capable of a 7 to 12 log reduction in the level of *L. monocytogenes*, therefore it is assumed that any ingredients added prior to the heat treatment should introduce minimal hazard and subsequent risk from *L. monocytogenes*.

Lysactone, rennet, starter culture and mould spores and added after pasteurisation. Supplier guarantees and certificates of analysis are used as support documents to provide assurance of the quality of these ingredients. Ryser & Marth (1999) previously reported that rennet of animal origin may occasionally be contaminated by animals carrying *L. monocytogenes* in the gut. However, in a review of published work, Ryser & Marth (1999) demonstrated that calf and bovine rennet are normally held in distribution long enough to ensure the coagulants are *L. monocytogenes*-free.

Microbial rennet extracts would normally be free of *L. monocytogenes* when manufactured, however if the product is not handled appropriately then it can become contaminated within the factory, where *L. monocytogenes* survival is enhanced compared with other rennet types (Ryser & Marth, 1999). Given these findings, the International Dairy Federation (IDF) has also considered adding rennet to the list of cheesemaking ingredients to be tested for *Listeria* spp. The commercially prepared concentrated freeze-dried starter cultures and mould cultures currently used should provide very little risk from contamination, although they are listed in the Hazard Audit Table (Table 4.4) for consideration.

4.3.1.2 Brie Food Safety Schemes

Factory 'A' had in place a HACCP-based Quality Assurance System prior to the commencement of this project. The scheme included both food safety and product quality elements. This project is focussed on food safety, therefore slightly modified versions of the Flow chart and Hazard Audit Table are presented in Table

4.3 and 4.4 respectively. The Flow Chart presents all elements of the process, with the Factory-designated Critical Control Points highlighted in bold. The monitoring procedures, specifications and corrective action for each CCP are shown in the Hazard Audit Table (Table 4.4).

Not all identified CCPs relate specifically to the potential hazard of *L. monocytogenes* contamination, but any step highlighting the risk of microbial contamination or growth is included here for consideration. These steps may represent possible pathways by which the product may become contaminated during manufacture and where a control failure may present a food safety hazard. The appropriateness of the identified CCPs for the control of the *L. monocytogenes* hazard will be discussed in the following sections, with objective analysis provided by risk assessment outcomes.

4.3.2 Brie manufacture: Process Risk Model

A PRM was conducted initially to predict possible *L. monocytogenes* growth during Brie manufacture. The Brie manufacture process was studied and characterised as outlined in Chapter 2. The resulting distributions of measured production parameter values are shown for each manufacture stage: Production (Figs 4.2a-c); Draining (Figs 4.11a-c); Maturation (Figs 4.13a-c); Final product attributes (Figs 4.22), and Storage and Distribution (Figs 4.23). In all cases a normal distribution was able to fit the data adequately. Goodness-of-fit statistics (Chi Square and Kolmogorov-Smirnov) are presented in Appendix E. The input parameter values, and input distribution curves generated by the @RISK software for stochastic modelling are also shown in Appendix E. The predicted *L. monocytogenes* growth during each stage is presented graphically in the following sections, with tabulated values shown in Appendix E.

4.3.2.1 Parameter interactions

As stated in Chapter 2, it is important to recognise parameter inter-dependencies which may influence modelling process outcomes. Scatter plots were constructed and examined to reveal possible interactions between temperature and pH during the initial stage of the cheesemaking process. Temperature affects growth of starter organisms and thus the production of lactic acid to lower the pH. Temperature and time were plotted against pH to determine the nature (if any) of the relationship between them (Fig 4.3a and 4.3b).

Table 4.3 - Process Flow Chart for 1 kg Brie manufacture

⇒ Product transfer □ Inspection ○ Operation

Factory-designated Critical Control Points indicated in **bold**

□	**Raw Milk Receival**
□ ○	Standardisation of milk
○	Thermalising
○	Addition of starter
○	Pre-ripening
○	Cooling
□ ○	**Pasteurisation**
⇒	Pump into Fill tank
□ ○	**Addition of ingredients (Lysactone, starter, mould, calcium chloride)**
○	Ripening
□ ○	**Fill into Bassines / Addition of rennet / Check pH**
□ ○	Determine prise
□	Calculation of cutting time
□ ○	Cut
□ ○	Stir
□ ○	Whey off
○	**Hooping**
○	Distribution of curd

O	Turn 1
⇒	Transfer to Draining Room
O	Draining
□	1.5 hr pH, moisture and temperature check
□	2.5 hr pH and temperature check
□ O	Turn 2 / 4 hr pH and temperature check
□	6 hr check, pH, moisture and temperature
□	8 hr check pH and temperature
O	Turn 3
□ O	Dehooping
□ O	**Brining**
⇒	Transfer to Drying Room
□ O	**Spraying with mould**
O	Turn
⇒	Transfer to maturing room
□ O	**Maturing**
O	5 day turn
O	7 day turn
⇒	Transfer to Wrapping Coolroom
O	**Wrapping**
⇒	**Transfer to Warehouse**

Adapted from J. Moffit (*pers comm.*, 1998)

Table 4.4 - Hazard Audit Table for 1 kg Brie Manufacture

Step number / Operation	Potential Hazard	Critical Control Point	Preventative Control and Monitoring Procedure			Corrective Action
			Monitoring	Specification	Frequency recorded, responsibility	
1. Raw milk receival	Poor quality milk		pH Acidity	6.60 - .670 0.10 – 0.17	Before batching milk to cheese room Manufacture book Pasteuriser Operator	Do not batch milk, contact Departmental Supervisor
2. Pasteurisation	Survival of pathogens	Temperature Flow diversion Phosphatase Flow rate	Temperature chart Temperature chart Laboratory records Pasteuriser charts	72.5 to 74°C Divert at 72.5°C < 10µg ρnitrophenol/mL 12,000 L/hr	Continuous recording Daily diversion check at startup Once per day Pasteuriser Operator Once per week	Diversion Do not pasteurise until diversion works Stop production, check pasteuriser function, place product on hold and test cheese. Contact maintenance supervisor
3. Addition of ingredients	Microbial contamination Traceability of ingredients	Certificate of Analysis Record batch codes	At receival Preparation / addition of ingredients	Raw material specifications All codes must be recorded		Do not use till Certificate provided Trace back possible codes from Dry Store records
4. Hooping	Contamination of product	Hygiene of operators and environment	Analysis of product after brining Visual observation	See Product specs	Every batch Lab records Cheesemaker and Lab Operator	Increase number of swabs to identify problem. If product out of spec do not release.

Step number / Operation	Potential Hazard	Critical Control Point	Preventative Control and Monitoring Procedure			Corrective Action
			Monitoring	Specification	Frequency recorded, responsibility	
5. Brining	Microbial or extraneous matter contamination	Hygiene of brine and equipment	Microbiological analysis of brine	See Brining room manual	Weekly Lab records Brine room operator, Lab operator	Check/adjust pH of brine, filter and replace if necessary
6. Spraying with mould	Microbial contamination Destruction or inhibition of mould	Compressed air quality Temperature of salt addition	Sterilisation of air filters Before adding mould to bottle	Routine Test Manual Room temperature	Weekly – Lab records Maturing room operator, Lab operator Each bottle - Not recorded Maturing room operator	 Cool to room temperature before adding mould
7. Maturing	Microbial contamination	Effective hygiene of personnel and maturing rooms	Microbiological analysis of product at wrap Environmental monitoring	Coliforms < 100/g Coliforms < 100/g	Each batch – Lab records Wrapping leading hand, Lab operator Weekly – Lab records Maturing room operator, Lab operator	Clean all appropriate areas Clean all appropriate areas
8. Wrapping	No traceability Trade description incorrect	Manufacture day code and run added to back label sticker Reorder of packaging	Before wrapping – at setup of date stamping machine	Product specification	Each batch – Rejects wrapping book Hand wrapping operator	If code incorrect record to ensure all relevant people are informed
9. Cool Storage	Product spoilage and growth of pathogenic microorganisms	Effective hygiene and sanitation practices Temperature	GMP Monitor and control coolroom temperature	Clean sanitary equipment and practices. Target 4°C maximum, range 2 to 6°C	Daily Coolroom temperature book Production manager	Reclean and sanitise all equipment and coolroom. Adjust temperatures accordingly

Adapted from J. Moffit (*pers comm.*, 1998)

The broader scatter of points in Fig 4.3a indicated that pH was time-dependent, rather than temperature-dependent. Thus, a number of factors were assumed to affect the acid development in the cheese. These include syneresis, with whey drainage and the resultant entrapment and concentration of the lactic acid within the curd, as well as the direct result of starter bacteria growth due to temperature.

4.3.2.2 Brie production profile

Brie production (0-4 hr)

The Brie production stage is considered to commence as the liquid milk emerges from the pasteuriser and heat exchanger, and enters the fill tank at a temperature of 39.2 ± 0.4°C (n = 94) and pH of 6.45 ± 0.11 (n = 92). The pH is standardised to 6.40 ± 0.04 (when necessary) through addition of Lysactone. This is reflected in Table 4.2, where the standard deviation for the milk pH decreases after standardisation (i.e. from the first to the second step in the process). The *Penicillium* mould and starter culture are added to the milk in the fill tank, but this does not immediately affect pH, as there appears to be a lag phase prior to the commencement of starter growth, and usually high levels are needed before an effect on pH is observed (Wan *et al*, 1997). Forty-five minutes later the milk is pumped into bassines (plastic tubs with a capacity of 150 L) at a temperature of 38.2 ± 1.2°C (n = 92) and pH of 6.38 ± 0.05 (n = 92) with rennet added simultaneously. The curd forms after several minutes, it is stirred and cut 45 minutes after the milk is filled, the cheese is hooped at a temperature of 37.3 ± 1.3°C (n = 93) and pH of 6.29 ± 0.12 (n = 92). The cheese is moved into the draining rooms where the whey is allowed to drain. When the draining rooms are full, the plastic air curtains are shut, trapping the heat from the fresh curd and slowing the rate of cooling. After two hours in the draining room, the cheese is turned for the first time, allowing it to form an even shape and aiding in an even expulsion of whey. The temperature remains high 32.9 ± 1.8°C °C (n = 99), and the pH falls to 5.67 ± 0.16 (n = 99).

The probability of potential *L. monocytogenes* growth for the initial four hours of the production is shown in Fig 4.4a, with the maximum outcome being a ten-fold increase (95^{th} percentile = log 0.97). The sensitivity analysis (Fig 4.4b) shows that temperature is the controlling factor for growth during this stage, with a correlation factor (c) of -0.97, with pH (c = 0.07) and salt concentration (c= -0.05) having little influence.

Figure 4.2a - Mean temperature of Brie production (—), upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software

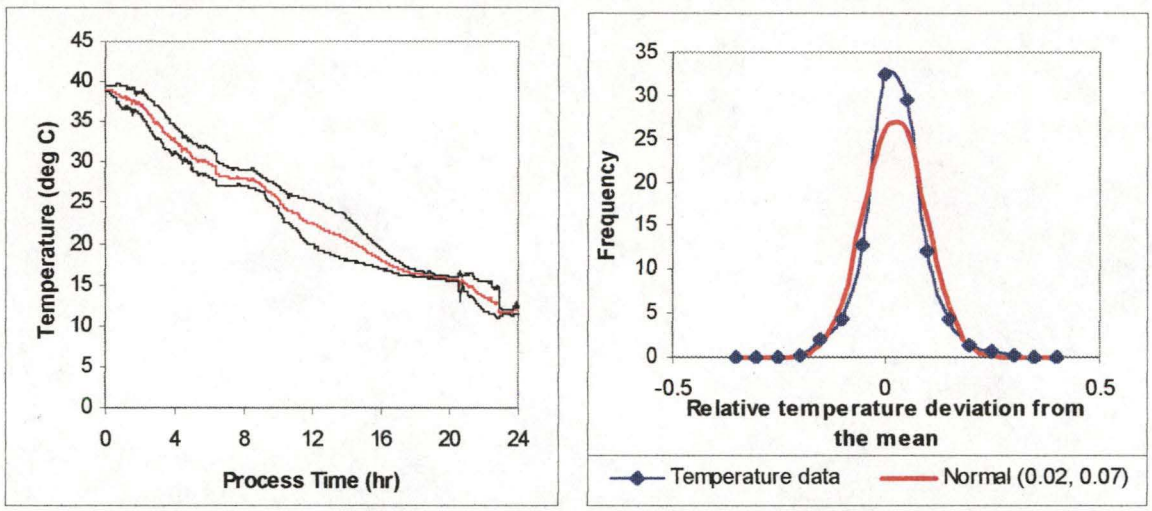


Figure 4.2b - Mean pH values for Brie production (—), upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software

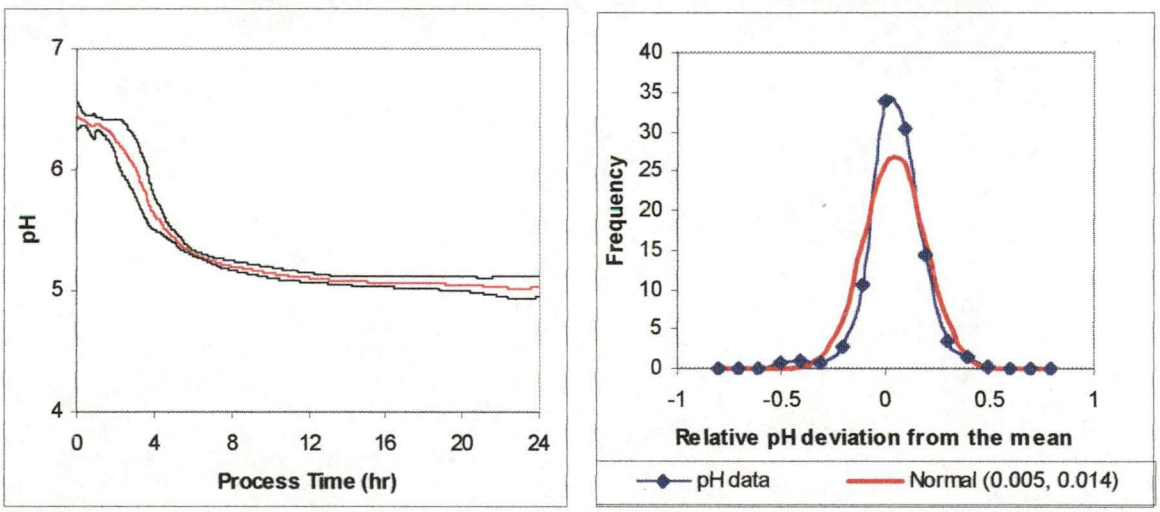


Figure 4.2c - Calculated salt concentrations for Brie production (—), upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software

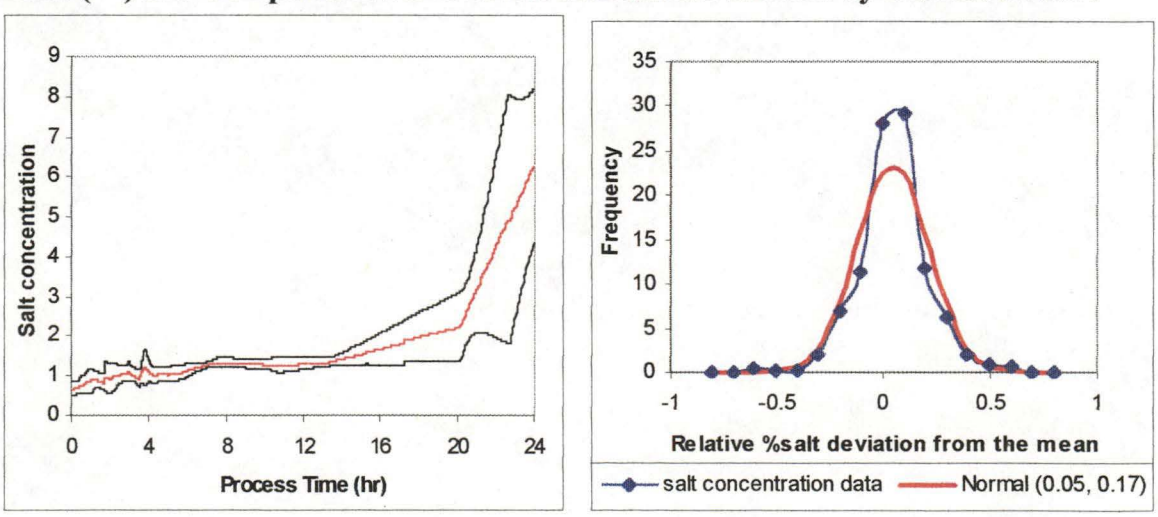
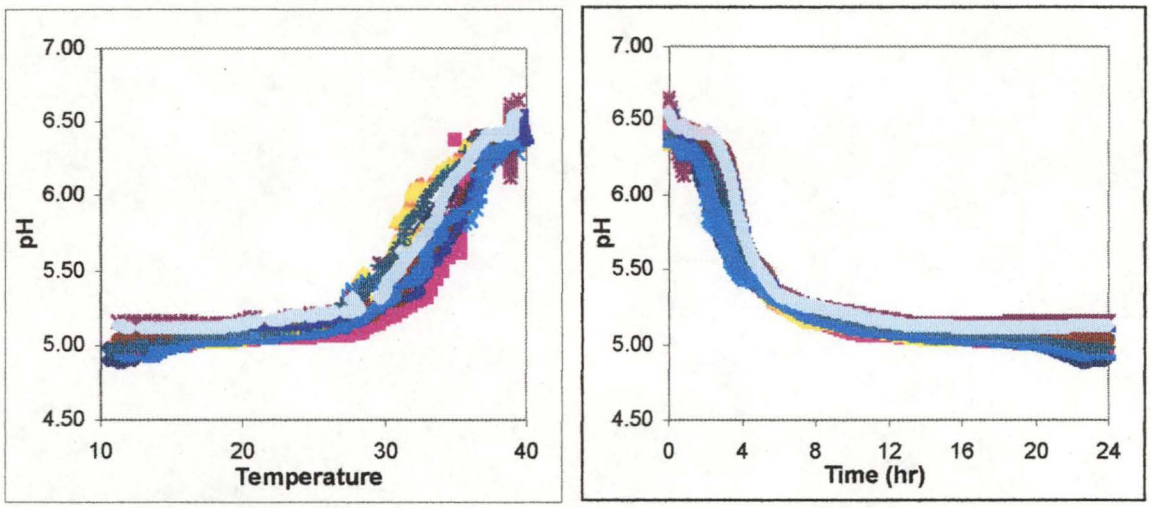


Figure 4.3 - Scatter plots to detect parameter interactions during Brie production stage

4.3a Temperature and pH **4.3b Time and pH**



Brie production (4-8 hr)

During hours 4-8 of Brie production, lactic acid development becomes significant, decreasing the pH to a level where it becomes the main factor controlling *L. monocytogenes* growth (c = 0.75) (Fig 4.5b). The cheese is turned after six hours, with the curd pH having dropped to 5.32 ± 0.02 (n = 10), and temperature to 30.0 ± 1.7°C (n = 10). The a_w values also decrease, reflected as an increased correlation with salt concentration (c = 0.19). Temperature remains significant (c = -0.57), again showing a negative correlation, as it remains above the optimum for part of the four hours. The maximum predicted *L. monocytogenes* growth is a 1 log increase (95^{th} percentile = log 0.94), but the most likely outcome is an increase of log 0.8 - 0.9 (Fig 4.5a) (50^{th} percentile = log 0.87).

Brie production (8-12 hr)

The combination of lowered pH and temperature during the 8-12 hours of the production stage results in a predicted maximum log 0.85 increase (Fig 4.6a). Similar to the previous four hours, the most important determinant of growth inferred from the sensitivity analysis (Fig 4.6b) is pH (c = 0.76), followed by temperature (c = 0.57), with salt concentration remaining of little significance (c = -0.13). The most likely predicted outcome at this stage is a 0.67 log increase (50^{th} percentile = log 0.65, 95^{th} percentile = log 0.73), or approximately two generations of *L. monocytogenes* growth.

Figure 4.4 - Modelled probability of potential *L. monocytogenes* growth during 0 - 4 hour stage of production and analysis of sensitivity to input variables

4.4a **4.4b**

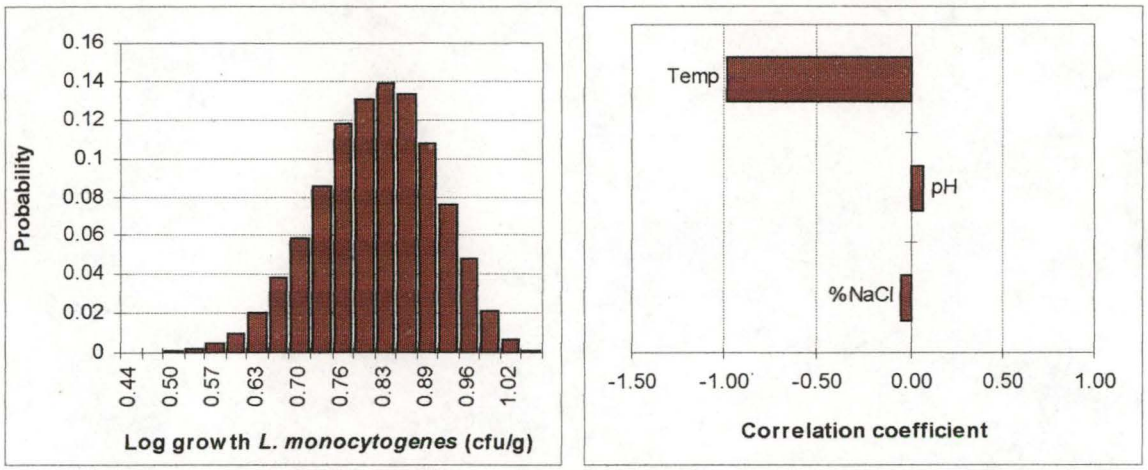


Figure 4.5 - Modelled probability of potential *L. monocytogenes* growth during 4 - 8 hour stage of production and analysis of sensitivity to input variables

4.5a **4.5b**

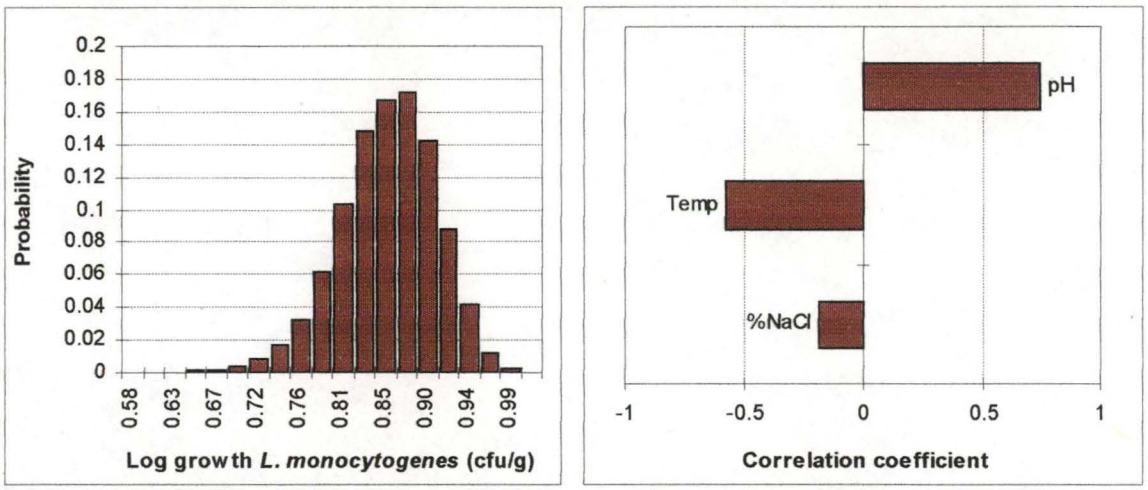


Figure 4.6 - Modelled probability of potential *L. monocytogenes* growth during 8 - 12 hour stage of production and analysis of sensitivity to input variables

4.6a **4.6b**

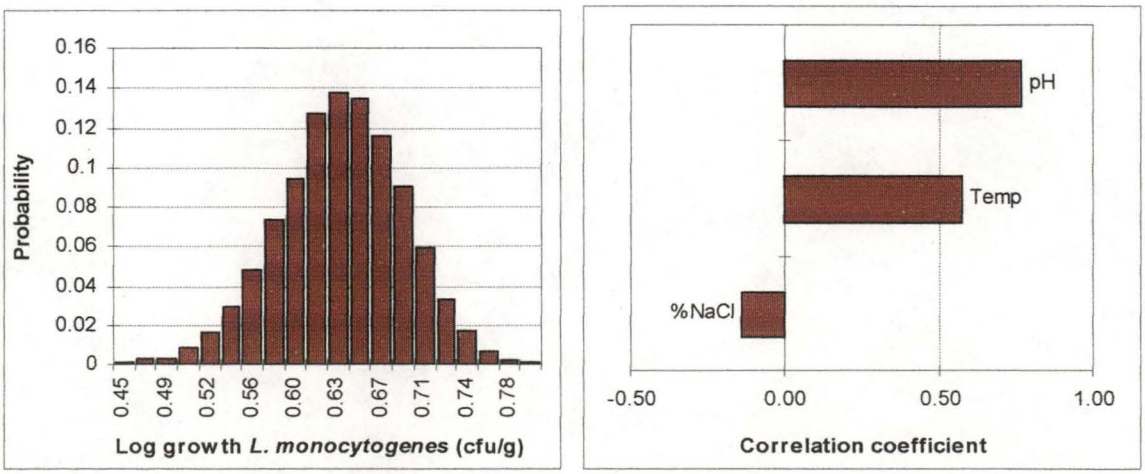


Figure 4.7 - Modelled probability of potential *L. monocytogenes* growth during 12 - 16 hour stage of production and analysis of sensitivity to input variables

4.7a **4.7b**

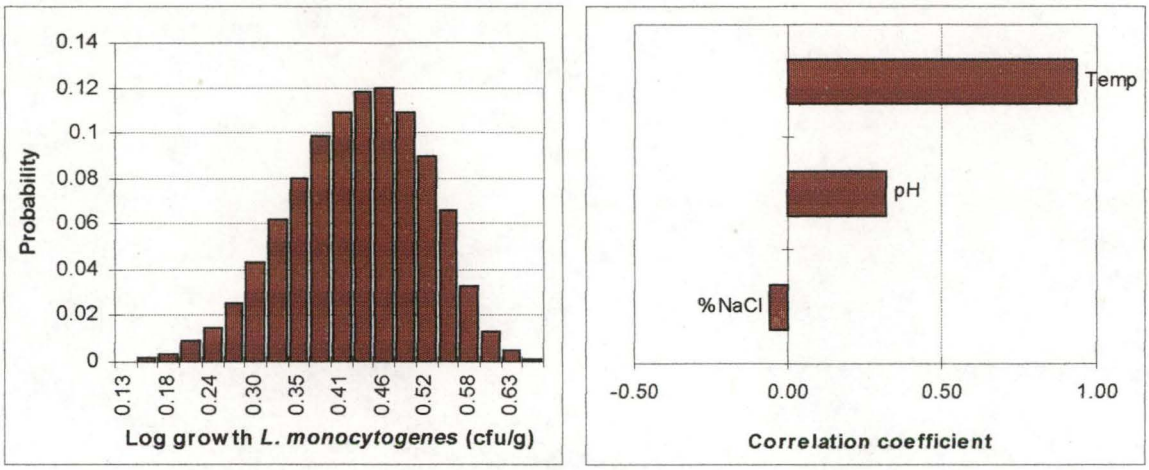


Figure 4.8 - Modelled probability of potential *L. monocytogenes* growth during 16 - 20 hour stage of production and analysis of sensitivity to input variables

4.8a **4.8b**

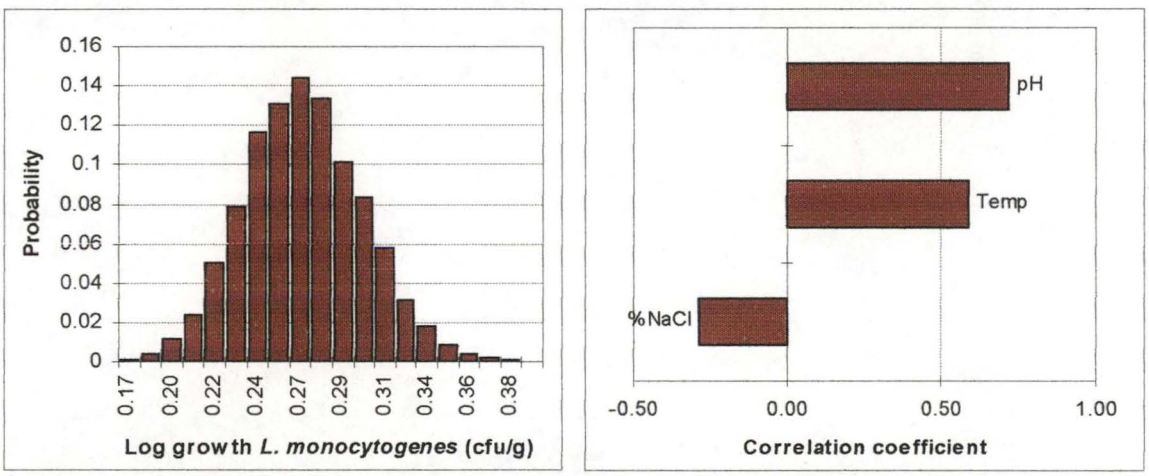


Figure 4.9 – Modelled probability of potential *L. monocytogenes* growth during 20 - 24 hour stage of production and analysis of sensitivity to input variables

4.9a **4.9b**

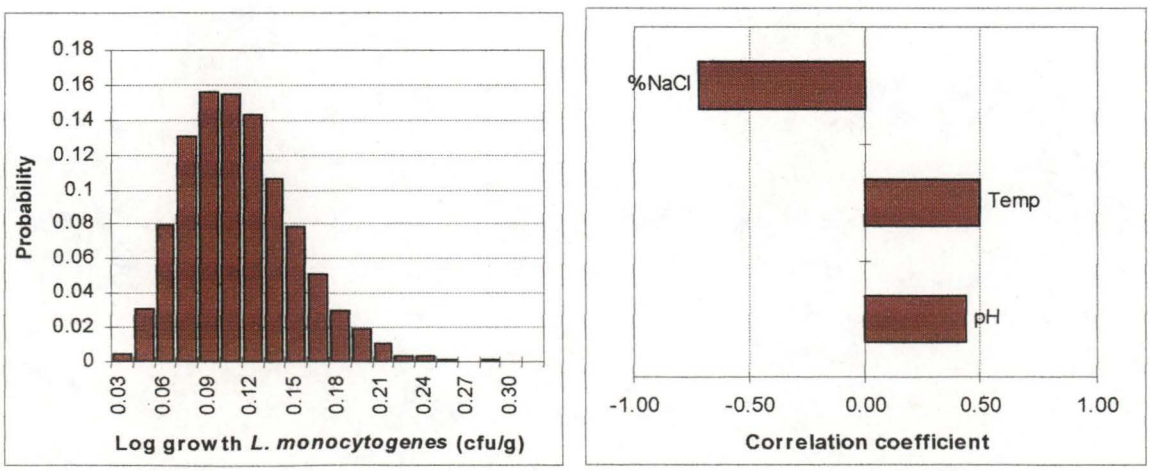
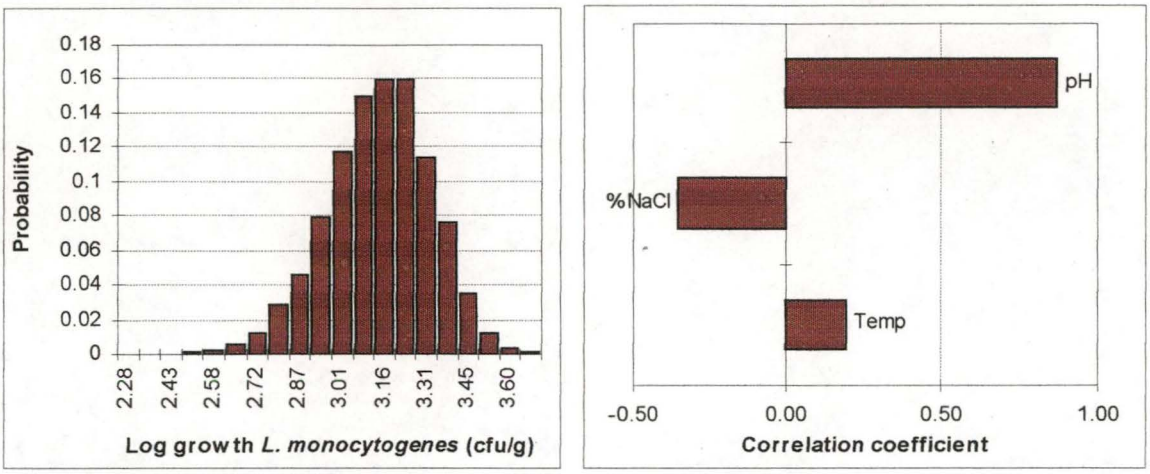


Figure 4.10 – Modelled probability of potential *L. monocytogenes* growth during all stages of production (0 – 24 hr) and analysis of sensitivities to input variables

4.10a **4.10b**



Brie production (12-16 hr)

After approximately 12 hours, the production shift ceases for the day. The production room exhaust fans are switched off and the ambient temperature of the production room drops (results not shown), with a resultant increased variability in the cheese cooling rate, revealed in Fig 4.2a by a greater standard deviation between hours 12-16. The sensitivity analysis for this four hour period (Fig 4.7b) shows that temperature again becomes the most significant factor in controlling *L. monocytogenes* growth (c = 0.94). The rate of acid production slows as most of the whey has drained from the cheese at this time, and starter bacteria growth becomes inhibited by the pH (5.10). However, due to the dominant effect of temperature, the correlation with pH decreases (c = 0.32). Salt concentration has very little influence (c = -0.06). The modelled *L. monocytogenes* growth during these four hours is much less than the previous stages (Fig 4.7a), with less than 2 generations of growth predicted (50^{th} percentile = log 0.45, 95^{th} percentile = log 0.58).

Brie production (16-20 hr)

During the 16-20 hour stage of the production process, the combination of reduced pH and temperature leads to the prediction that one generation of growth is the most likely outcome (Fig 4.8a and 4.8b) (50^{th} percentile = log 0.27, 95^{th} percentile = log 0.33). All three parameters exert an influence on potential *L. monocytogenes* growth, with pH the most significant (c = 0.72), followed by temperature (c = 0.59), and salt concentration (c = -0.28).

Brie production (20-24 hr)

The cheese remains in the draining room until hour 20 of production, when it is de-hooped. At this stage the cheese temperature is 15.3 ± 1.2°C (n = 98) and pH 5.04 ± 0.06 (n = 100). The cheese is immersed into brine solution (~20% salt) for 90 minutes. This significantly lowers the cheese water activity (increases the equivalent salt concentration), shown in Fig 4.2c, and demonstrated by salt concentration becoming the most significant factor for the first time during the production stage (Fig 4.9b) (c = -0.71). Along with the increased salt concentration, temperature (c = 0.50) and pH (c = 0.44) also provide significant hurdles to *L. monocytogenes* growth. As a result, predicted growth during these four hours is greatly reduced (Fig 4.9a) (50^{th} percentile = log 0.12, 95^{th} percentile = log 0.19), The Brie is removed from the brine after 90 minutes and moved to the draining room, corresponding to the next process step.

Brie production - totals

Consideration of the predicted growth for the entire 24 hr of the production stage shows that the total amount of *L. monocytogenes* growth possible is in excess of 3 log cycles (Fig 4.10a) (50^{th} percentile = log 3.19, 95^{th} percentile = log 3.46). The sensitivity analysis (Fig 4.10b) illustrates that pH is the most significant parameter correlating to *L. monocytogenes* growth (c = 0.87).

4.3.2.3 Brie draining profile

The 24 hours following brining were defined arbitrarily as the draining stage. The temperature (Fig 4.11a) does not vary significantly during this stage, due to the cheese being kept in a constant temperature room. The pH is also very constant (Fig 4.11b) as the growth of the starter organisms is retarded by the low pH and the high salt concentration resulting from the brining process. The water activity of the cheese is lowered by the brining process to 0.964 ± 0.011 (n = 10) (Table 4.1) after which it emerges from the brine and is taken to the draining room, where the excess brine drains from the cheese. It can be seen from Fig 4.11c that at the start of this stage the salt concentration continues to increase for several hours after the cheese has been removed from the brine. This is possibly due to the evaporation of moisture from the surface of the cheese, leaving the remaining salt to be concentrated.

Figure 4.11a - Mean temperature of draining process step (—), with upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software

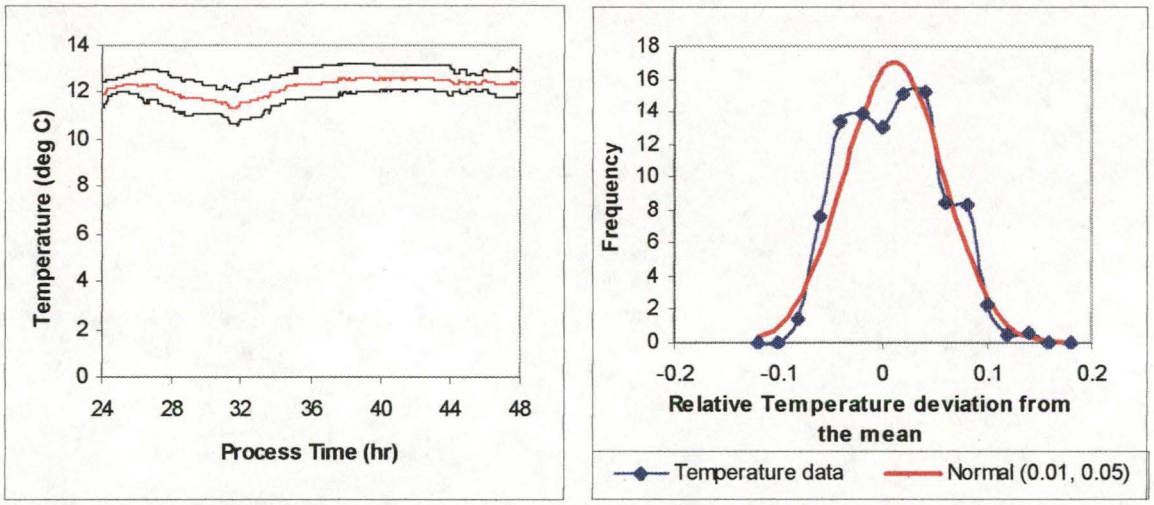


Figure 4.11b - Mean pH values for draining process step (—), with upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software

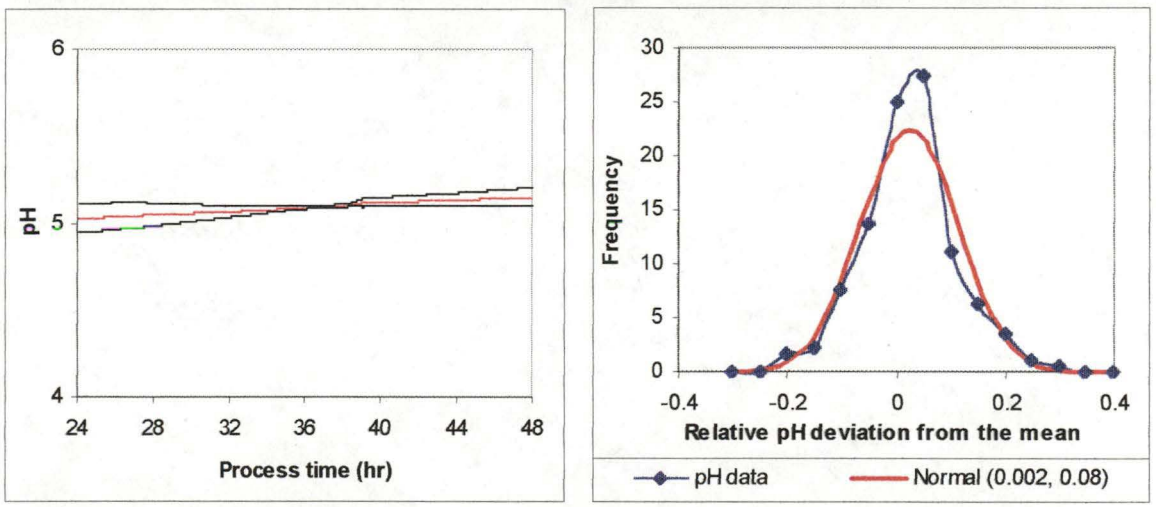
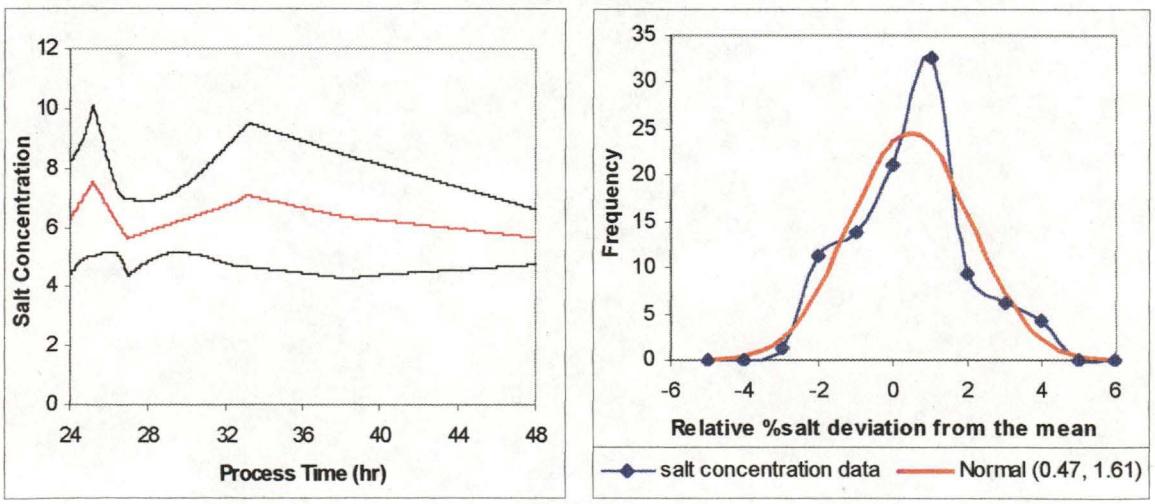


Figure 4.11c - Calculated salt concentrations for draining process step (—), with upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software

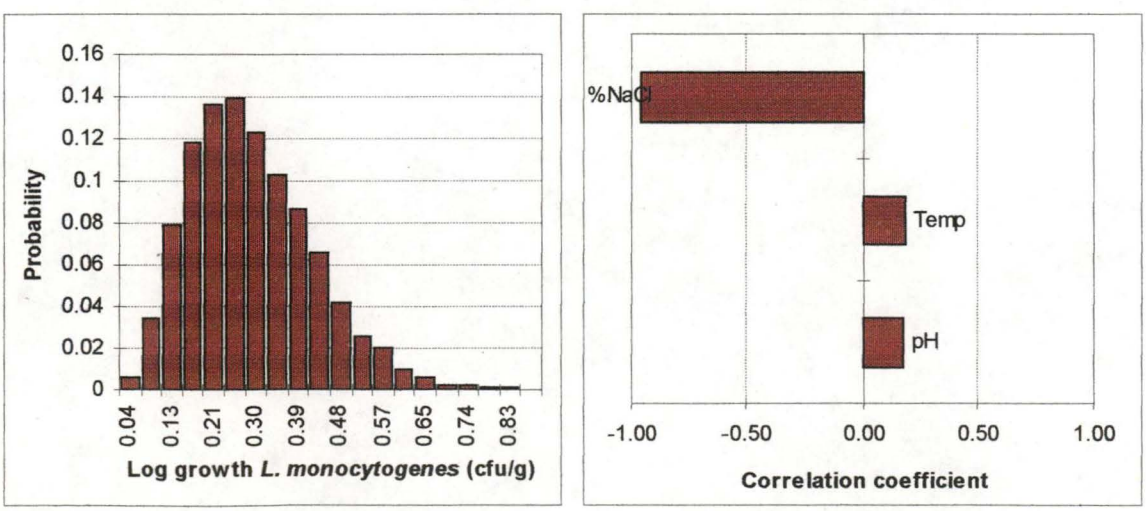


Once the cheese has drained for approximately 15 hours, a culture of *Penicillium* mould is added to the surface of the cheese by spraying spores with a compressed air spray pack. At this stage the water activity of the cheese is 0.959 ± 0.008 (n = 10), the pH is 5.10 ± 0.01 (n = 10) and temperature is 12.6 ± 0.6°C (n = 10) (Table 4.2). Early the next morning the cheese is moved to a temperature-controlled maturing room, approximately 48 hours into the process, corresponding to the next process step.

Due to the stability of process parameters throughout the draining stage, a single 24 hr time period was adequate to observe trends. However, the model outcomes for each 4 hr interval are presented in Appendix E. The potential *L. monocytogenes* growth during the 24 hr of draining is shown in Fig 4.12a. The most common outcome was calculated to be a log 0.32 increase (1 generation) (50^{th} percentile = log 0.30, 95^{th} percentile = log 0.55). However, the maximum calculated output was a log 0.92 increase. Salt concentration was the most limiting factor (Fig 4.12b) (c = -0.95), followed by temperature (c = 0.19) and pH (c = 0.18). Ryser and Marth (1987b) showed that *L. monocytogenes* survives an aqueous solution of 25.5% (w/v) NaCl, therefore it is unlikely that the organism will be completely eliminated by the brining process in that 24 hour period.

Figure 4.12 - Probability of *L. monocytogenes* growth and analysis of sensitivity to input variables during draining stage (24 - 48 hr)

4.12a **4.12b**



4.2.2.4 Brie maturation profile

The maturation stage is the longest in terms of duration (7 days) and has a profound influence on product quality. Biochemical reactions take place during the maturation stage, which alter the physical, chemical and sensory properties of the cheese (Davis, 1976). The cheese is placed in the maturing room, which runs at constant humidity and temperature (12-13°C). The normal distribution shown in Fig 4.13a shows the narrow range of temperatures around the mean, indicating that temperature control is very good within the maturing rooms. The cheese is turned twice during the maturation phase at Day 5 and Day 7, allowing an even development of the surface *Penicillium* mould. The mould growth becomes visible on the surface during the last two days of the maturation stage, after the last turn. Corresponding to this is a rise in surface pH of the cheese, as shown in Fig 4.13b. A few hours before the cheese is to be packaged, it is moved to the coolroom. After wrapping the product is placed into the large coolroom ready for distribution.

Davis (1976) defined four structural zones in Brie cheese, ie, the surface, rind, ripe zone and unripened core. Surface and rind characteristics result from rapid growth of yeasts and micrococci after brining, followed by the outgrowth of *P. camemberti* spores. The cheese ripe zone results from diffusion of proteolytic enzymes into the cheese produced by the surface mould. After casein hydrolysis into water-soluble nitrogenous compounds, proteolysis continues through action of the starter organisms and the mould, which results in formation of low molecular weight nitrogenous compounds including amines and ammonia. During ripening, milkfat and lactic acid are degraded, and small amounts of CO_2 are produced. Upon further ripening, the unripened core decreases in size as the ammonia concentration increases in the rind and ripe zone.

Brie maturation – Day 1

The parameter correlations for the growth of *L. monocytogenes* in the first 24 hours of the maturation phase show that temperature (c = 0.63), pH (c = 0.56) and salt concentration (c = -0.48) all have a similar effect towards controlling *L. monocytogenes* growth (Fig 4.14b). The most likely outcome is a log 0.5 - 0.8 increase during this first day of maturation (50^{th} percentile = log 0.57, 95^{th} percentile = log 0.82), but there is potential for greater than a log increase (maximum = log 1.23) (Fig 4.14a).

Figure 4.13a - Mean temperature of maturation step (—), upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software

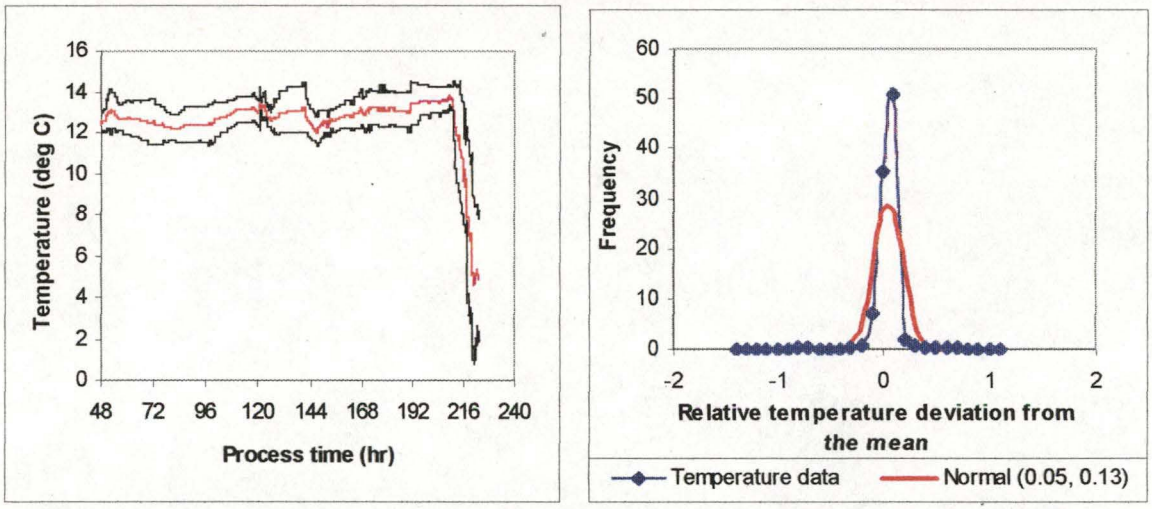


Figure 4.13b - Mean pH values for maturation step (—),upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software

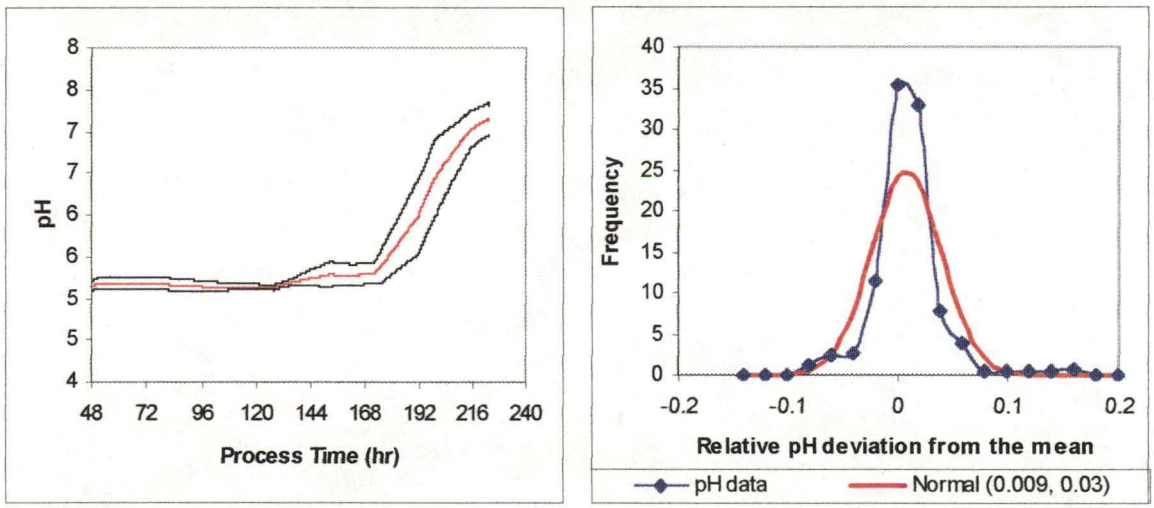
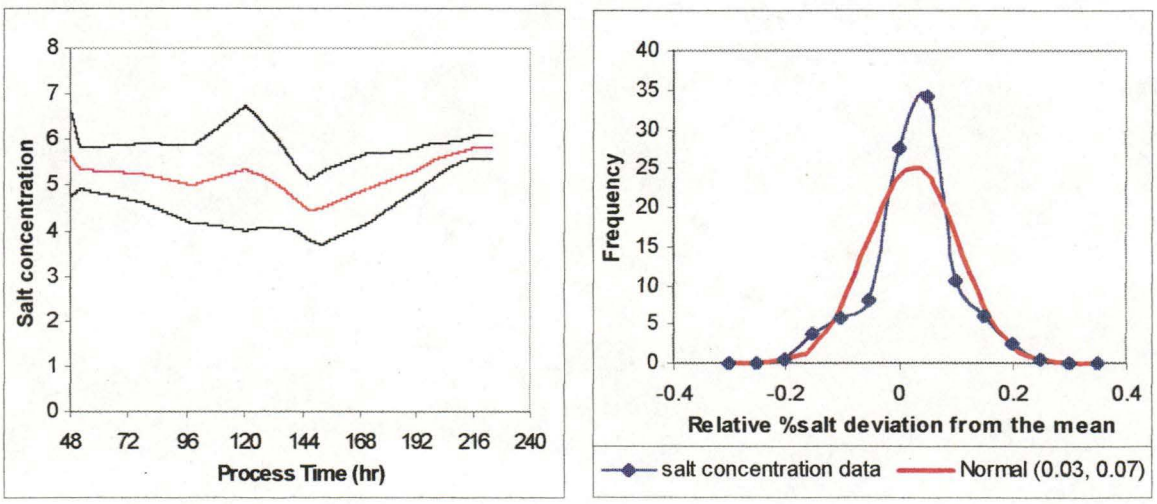


Figure 4.13c – Calculated salt concentrations for maturation step (—), upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software



Brie maturation – Day 2

The constant storage conditions of the maturation stage are reflected in the model outcomes. The predicted growth profile for Day 2 of maturation predicts a very similar scenario as Day 1 (Fig 4.15a). The most likely outcome is a log 0.4 - 0.6 increase (50^{th} percentile = log 0.55, 95^{th} percentile = log 0.80), however as for Day 1 there is a maximum predicted outcome of > 1 log increase (maximum = log 1.25). The parameter correlations are also of similar magnitude as the first day, however salt concentration has a slightly higher correlation (c = -0.59) (Fig 4.15b), due to the larger standard deviation (Fig 4.13c).

Brie maturation – Day 3

The a_w of the cheese rises to 0.967 ± 0.04 (n = 10), most likely due to the absorption of moisture from the humidity in the maturing room. However there is little change in pH, which remains steady at 5.14 ± 0.05 (Table 4.2). The change in a_w (and equivalent salt concentration) becomes most evident on Day 3 when the magnitude of the salt concentration correlation further increases (c = -0.81) (Fig 4.16b). Temperature (c = 0.46) and pH (c = 0.30) still have a significant effect on predicted *L. monocytogenes* growth. The growth predicted by the model is similar to the previous two days (50^{th} percentile = log 0.53, 95^{th} percentile = log 0.80) (Fig 4.16a).

Brie maturation – Day 4

The cheese is turned during Day 3, and the correlation for salt concentration remains high (c = -0.61), but temperature is predicted to have the most influence (c = 0.67) and pH is still significant (c = 0.36) (Fig 4.17b). The predicted growth profile for *L. monocytogenes* is similar in magnitude to the first three days (50^{th} percentile = log 0.63, 95^{th} percentile = log 0.89) (Fig 4.17a).

Brie maturation – Day 5

During the fifth day, the cheese is turned for the final time and the effect of the surface *Pencillium* mould becomes evident with the surface pH tending to rise to 5.43 ± 0.22 (n = 10) and pH becoming the most significant factor correlating to growth (c = 0.77) (Fig 4.18b). Temperature (c= 0.45) and salt concentration (c = -0.39) also have significant effect on the predicted amount of *L. monocytogenes* growth. With the conditions becoming more favourable for growth, the predicted

Figure 4.14 – Modelled probability of potential *L. monocytogenes* growth during Day 1 of maturation stage and analysis of sensitivity to input variables

4.14a **4.14b**

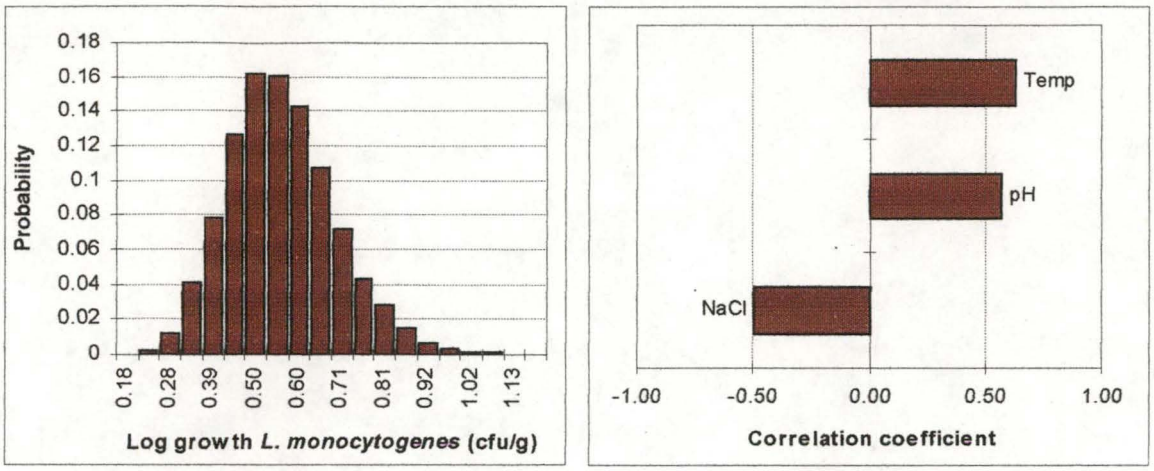


Figure 4.15 – Modelled probability of potential *L. monocytogenes* growth during Day 2 of maturation stage and analysis of sensitivity to input variables

4.15a **4.15b**

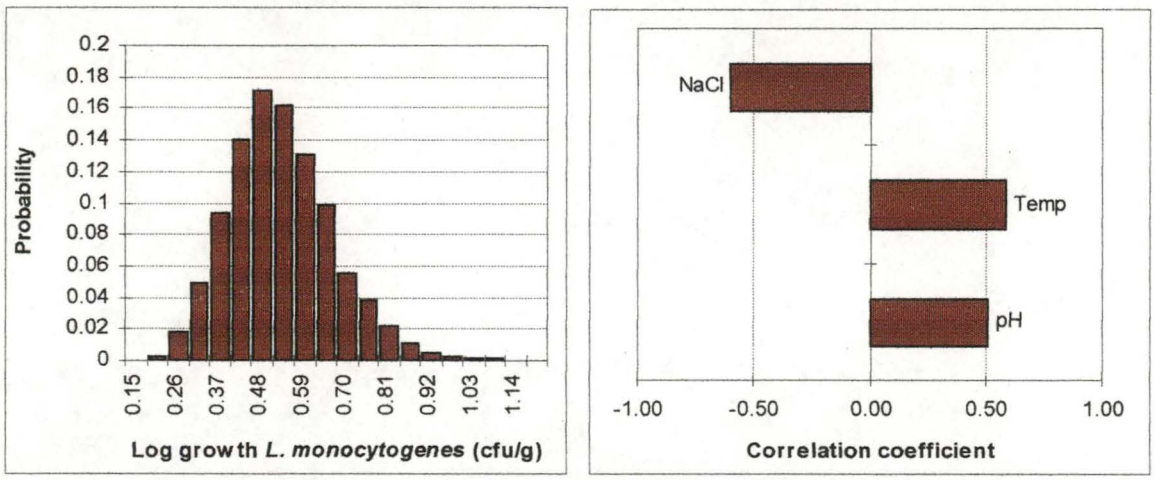


Figure 4.16 – Modelled probability of potential *L. monocytogenes* growth during Day 3 of maturation stage and analysis of sensitivity to input variables

4.16a **4.16b**

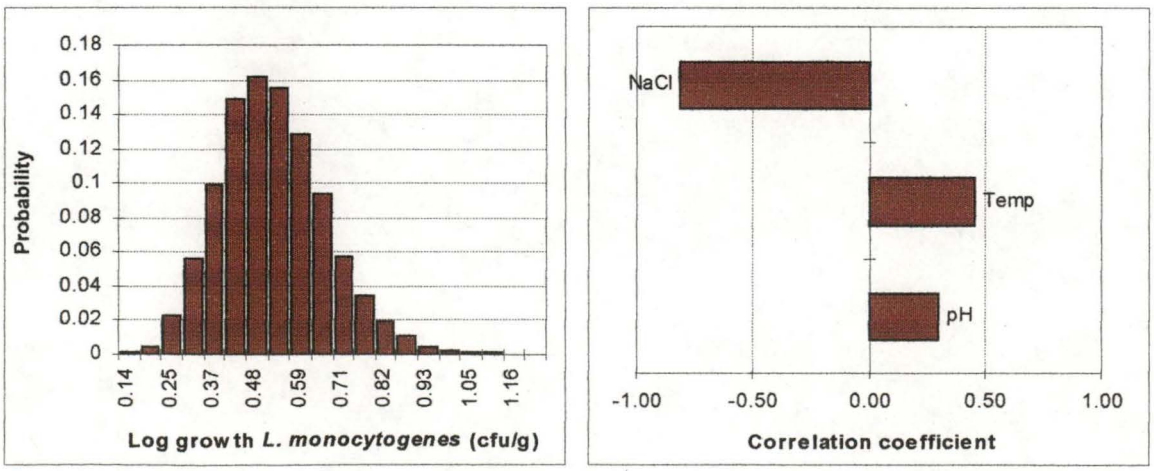


Figure 4.17 – Modelled probability of potential *L. monocytogenes* growth during Day 4 of maturation stage and analysis of sensitivity to input variables

4.17a **4.17b**

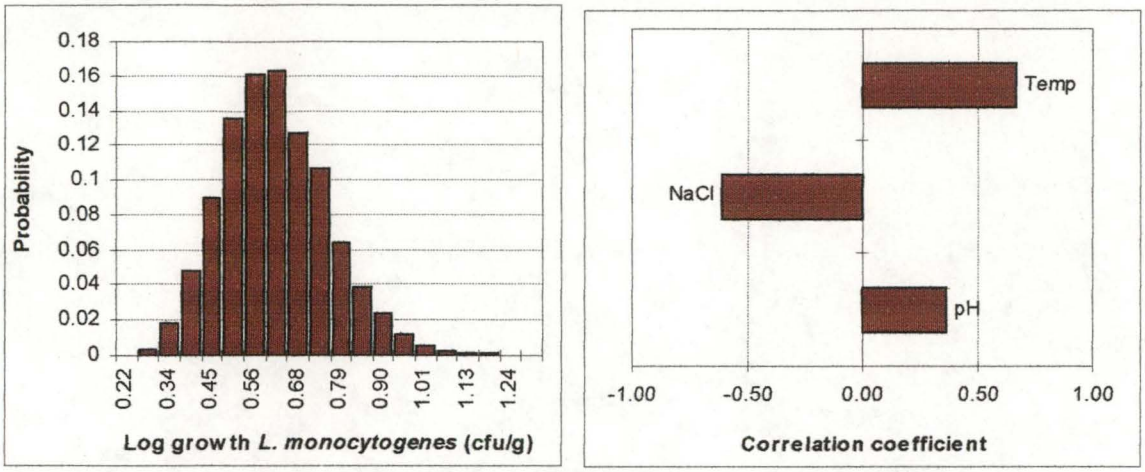


Figure 4.18 – Modelled probability of potential *L. monocytogenes* growth during Day 5 of maturation stage and analysis of sensitivity to input variables

4.18a **4.18b**

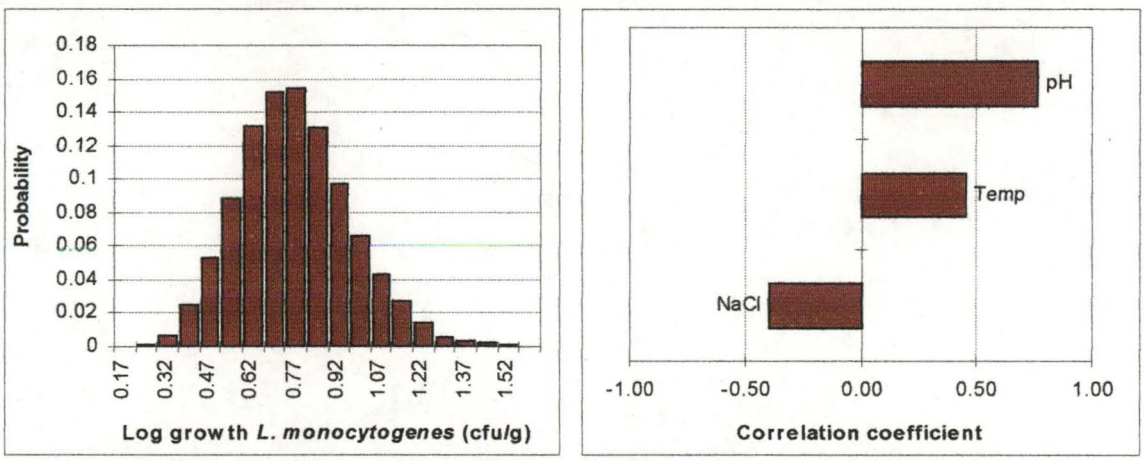


Figure 4.19 – Modelled probability of potential *L. monocytogenes* growth during Day 6 of Brie maturation and analysis of sensitivity to input variables

4.19a **4.19b**

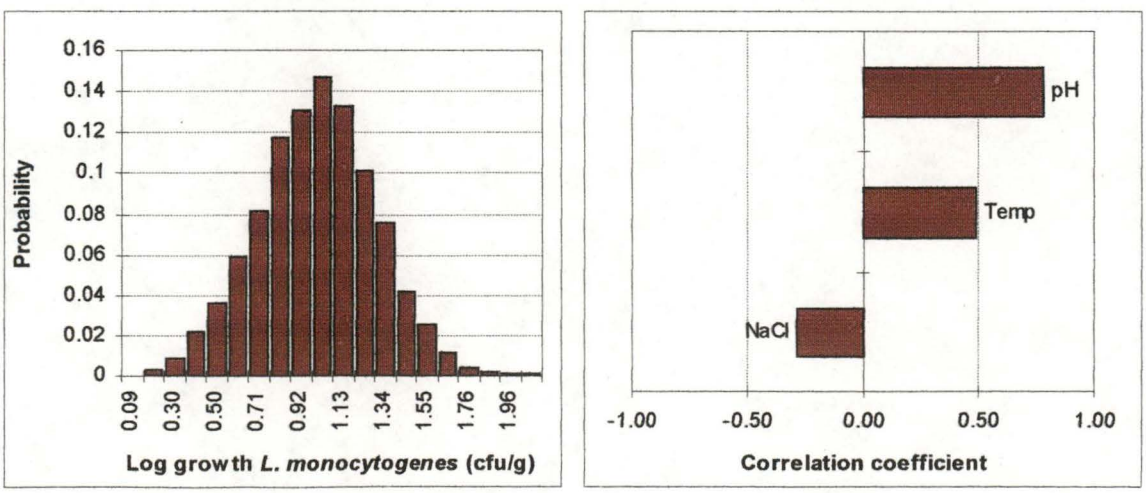


Figure 4.20 - Probability of potential *L. monocytogenes* growth and analysis of sensitivity to input variables during Day 7 of maturation stage

4.20a **4.20b**

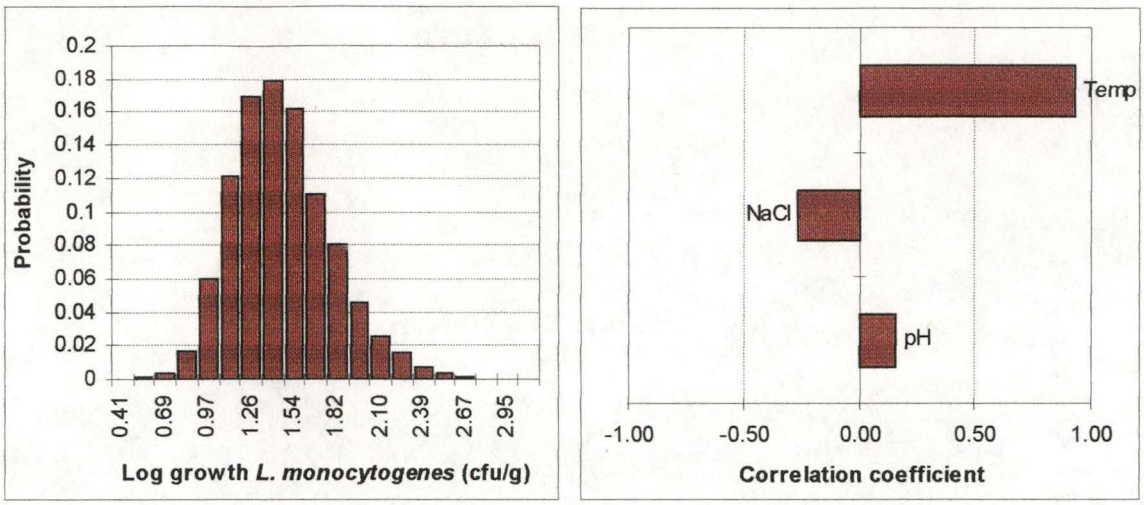
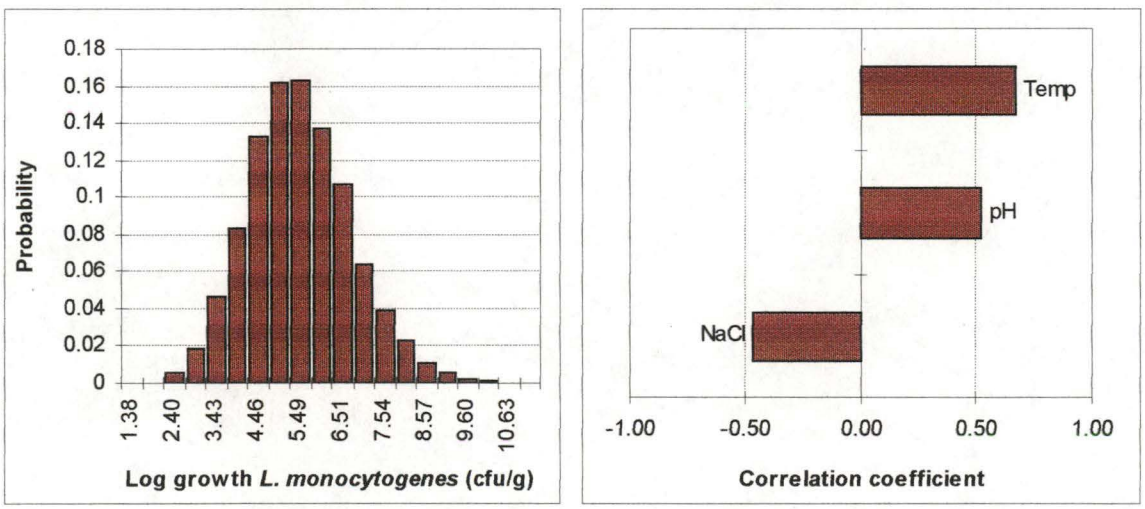


Figure 4.21 - Probability of potential *L. monocytogenes* growth and analysis of sensitivity to input variables during Brie maturation

4.21a **4.21b**



level of growth for this day is higher than on previous days (50th percentile = log 0.79, 95th percentile = log 1.14) (Fig 4.18a).

Brie maturation – Day 6

The further increase in pH during Day 6 of maturation results in more growth predicted during this 24 hour period, up to a 2 log increase possible (50th percentile = log 1.06, 95th percentile = log 1.53) (Fig 4.19a). The sensitivity analysis (Fig 4.19b) demonstrates pH (c = 0.79) to be the dominant controlling factor for predicted

L. monocytogenes growth, with temperature (c = 0.49) and salt concentration (c = -0.28) still significant. The model predicts a mean outcome of ten fold increase (log 1.05), but under the most favourable conditions a maximum output of 2.17 log is possible. Upon examination of the percentile values, this appears to be a rare outcome (50^{th} percentile = log 1.06, 95^{th} percentile = log 1.53).

Brie maturation – Day 7

Late on the 7^{th} day of maturation (9^{th} day since commencement of production), and several hours prior to wrapping, the cheese is placed in a 5°C coolroom. This cools the cheese slightly to ensure it does not significantly warm during the wrapping process. This ensures that temperature becomes the most significant factor affecting predicted *L. monocytogenes* growth (c = 0.93) (Fig 4.20b). The cheese is at a final pH of 7.03 ± 0.22 (n = 10), within the optimum range for growth, therefore the correlation is not as strong as it was previously (c = 0.16). The a_w value rises to 0.965 ± 0.001 (n = 10) at the time of wrapping, which significantly correlates to the predicted growth outcomes (c = -0.46). As the conditions within the cheese continue to become more favourable for *L. monocytogenes* growth, the predicted growth increases (50^{th} percentile = log 1.49, 95^{th} percentile = log 2.11) (Fig 4.20a), with a maximum predicted outcome of a log 3.23 increase possible during this 24 hour period.

Brie maturation – totals

The possible outcomes and correlations for the entire maturation process are shown in Figs 4.21a and 4.21b. The maximum *L. monocytogenes* growth predicted by the model was greater than 11 logs (50^{th} percentile = log 5.63, 95^{th} percentile = log 7.94). The sensitivity analysis (Fig 4.21b) demonstrates that all three parameters (i.e temperature, pH and water activity) significantly correlate to the model outcomes, temperature being the most dominant (c = 0.67), followed by pH (c = 0.53) and salt concentration (c= =-0.46). The finished Brie is wrapped in wax paper and an outside plastic layer. After wrapping, the cheese is placed on a pallet and stored in a large coolroom which cools the cheese down to about 4°C, as shown in Fig 4.13a. The cheese is then ready for further distribution within the state of Tasmania, and also exported to the Australian mainland.

4.2.2.5 Brie final product attributes

The Brie final product attributes are presented in Table 4.5. The number of lactic acid bacteria (LAB) was very constant in all cheese batches tested, at a level of ~ 10^9 cfu/g (n = 10). The standard plate count was more variable, indicating that the contamination rate in some batches was bigger than others. The number of yeasts and mould numbers found in the Brie were relatively constant. None of the Brie samples analysed for pathogenic bacteria in this study returned positive results. The distributions for pH and calculated salt concentrations (n = 53), and the fit provided by a normal distribution are shown in Fig. 4.22.

4.2.2.6 Brie storage and distribution

After the Brie is wrapped, it is placed in a large coolroom and stored for 24 hours until it is transported to its final destination. This product can be subjected to long periods of transit, as it is also exported to the mainland states of Australia. The temperature of transport and storage conditions was determined from factory records, from temperature loggers that had been included in cheese batches (Fig 4.23). The data was adequately described by a normal distribution (Fig 4.23). It can be seen that variability exists in the cheese temperature after initial placement in the coolroom. After approximately 36 hours the product is cooled to a constant temperature of around 2°C, with less variability as is represented through the middle portion of Fig 4.23. There were no measurements of pH and a_w taken during this process phase. Given the ability of the Normal distribution to accurately describe the data to this point, the assumption was made that the same distribution could also be used for this stage. Values from the final product analysis and the beginning of the shelf life analysis were used as starting and finishing values respectively for this stage in the modelling process. The input values and distributions are shown in Appendix E.

The model outcomes for the predicted growth of *L. monocytogenes* over the four days of distribution and storage are shown in Figs 4.24 - 4.27. The most dominant factor in controlling growth is storage temperature, as is shown by the large correlation values for each of the days. During this time the product has very favourable pH and a_w values for growth, as shown by the values at the time of wrapping (Table 4.5). Therefore storage temperature is the only method available to preserve the shelf life and minimise the risk of *L. monocytogenes* growth in the product.

Table 4.5 – Measured final product attributes for 1 kg Brie

	pH	a_w	LAB (log cfu/g)	SPC (log cfu/g)	Yeast (log cfu/g)	Mould (log cfu/g)
Mean	7.33	0.965	9.28	6.21	5.04	4.18
Std Dev	0.28	0.005	0.07	1.08	0.52	0.73
Maximum	8.02	0.976	9.36	8.20	5.71	5.20
Minimum	6.69	0.956	9.13	5.11	4.34	3.00

Figure 4.22 – Measured surface pH and calculated salt concentrations for final product and Comparison with Normal distribution selected by Bestfit software

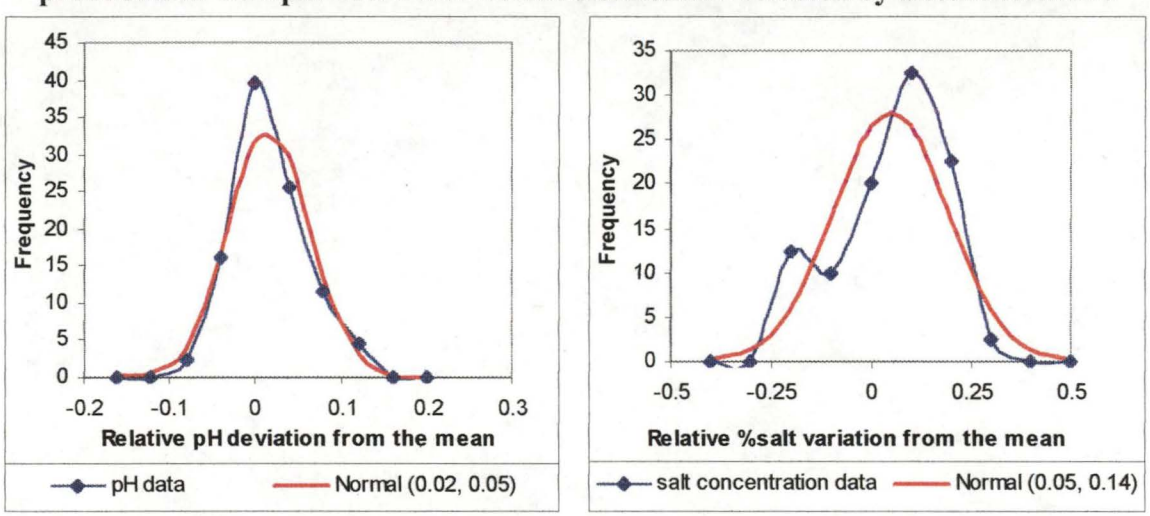


Figure 4.23 - Mean temperature values for coolroom storage and distribution (—),upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software

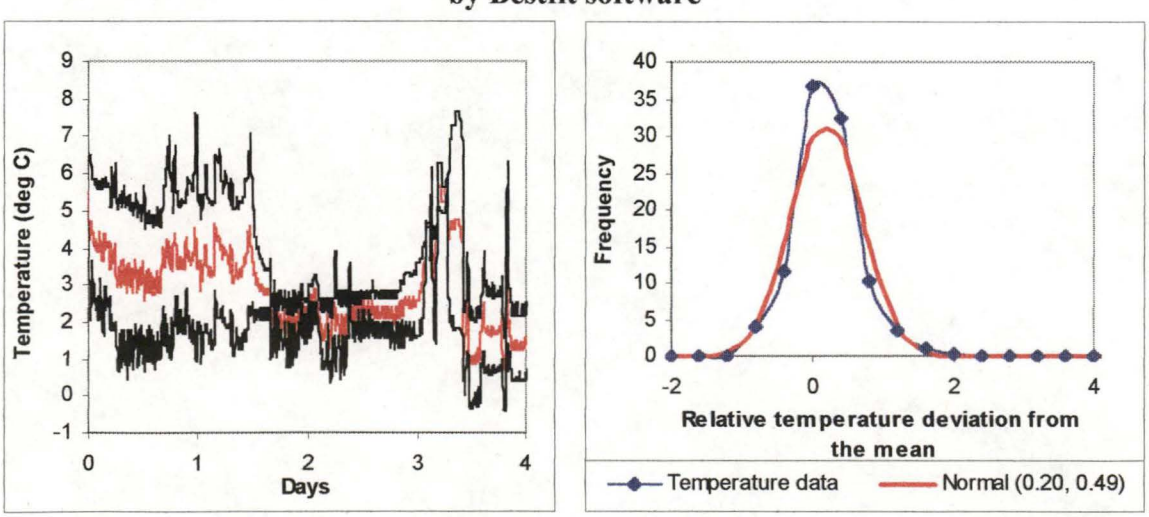


Figure 4.24 – Modelled probability of potential *L. monocytogenes* growth during Day 1 of storage and distribution and analysis of sensitivities to input variables

4.24a **4.24b**

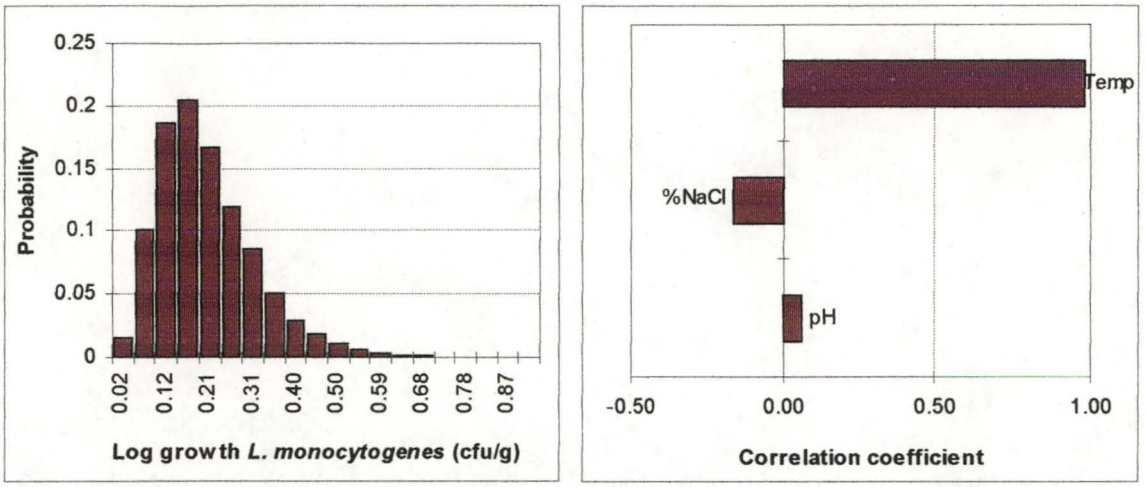


Figure 4.25 - Modelled probability of potential *L. monocytogenes* growth during Day 2 of storage and distribution and analysis of sensitivities to input variables

4.25a **4.25b**

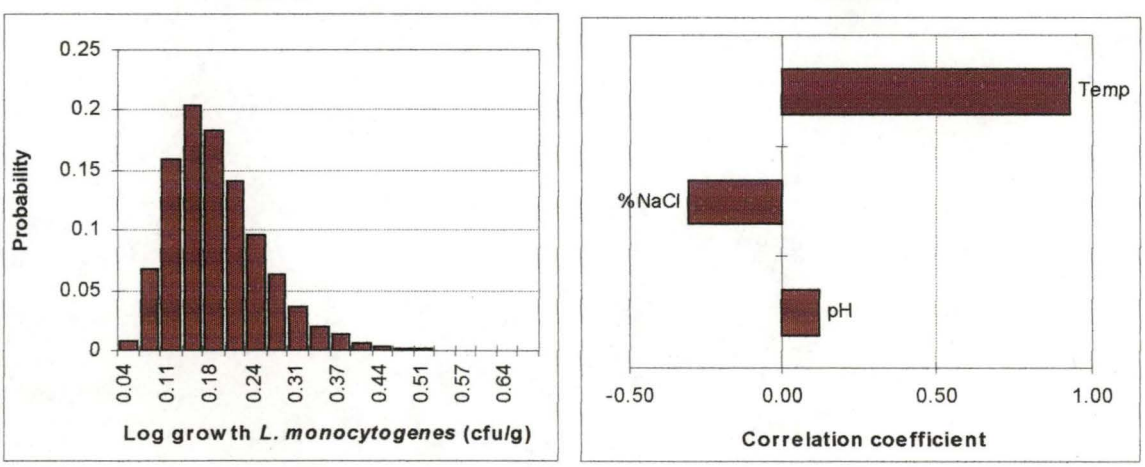


Figure 4.26 - Modelled probability of potential *L. monocytogenes* growth during Day 3 of storage and distribution and analysis of sensitivities to input variables

4.26a **4.26b**

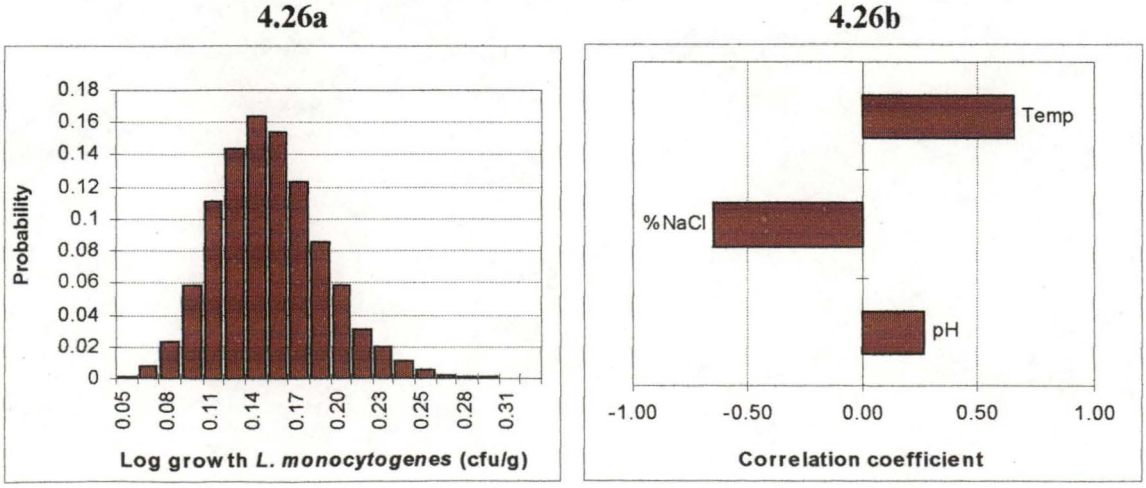


Figure 4.27 - Probability of potential *L. monocytogenes* growth and analysis of sensitivity to input variables during Day 4 of storage and distribution

4.27a **4.27b**

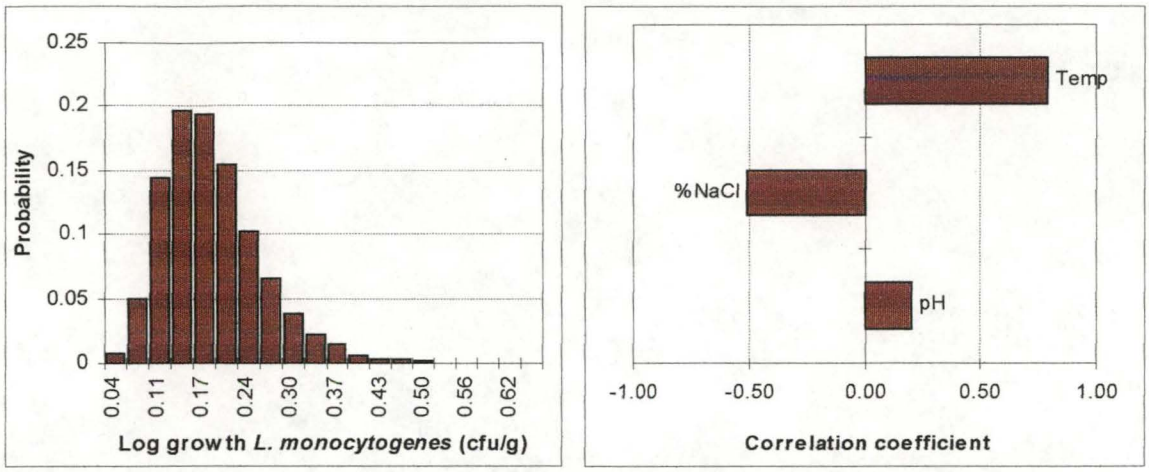
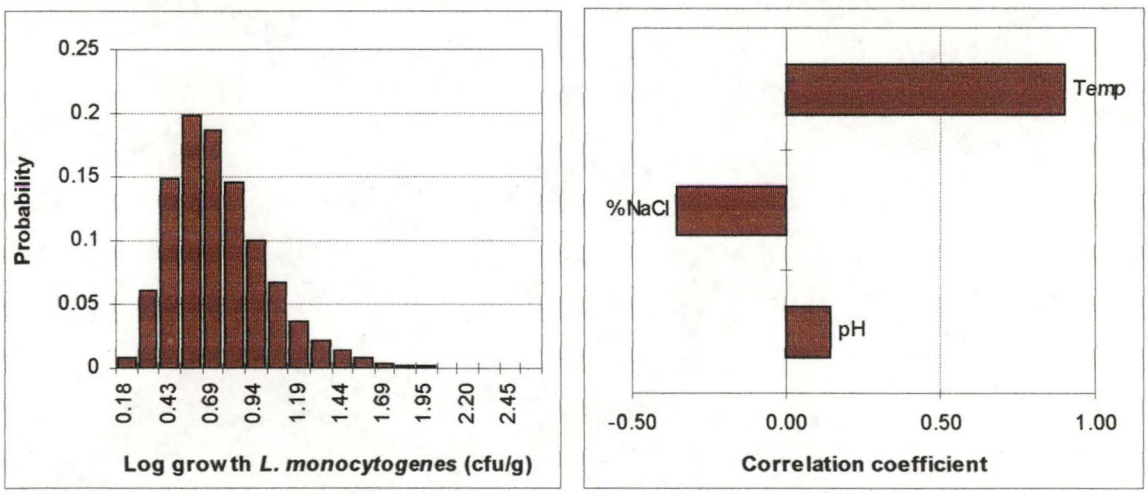


Figure 4.28 - Probability of potential *L. monocytogenes* growth and analysis of sensitivity to input variables during all storage and distribution phase

4.28a **4.28b**



The model outcomes for the predicted growth of *L. monocytogenes* over the four days of distribution and storage are shown in Figs 4.24 - 4.27. The most dominant factor in controlling growth is storage temperature, as is shown by the large correlation values for each of the days. During this time the product has very favourable pH and a_w values for growth, as shown by the values at the time of wrapping (Table 4.5). Therefore storage temperature is the only method available to preserve the shelf life and minimise the risk of *L. monocytogenes* growth in the product. It can also be seen that the variability of temperature during the first 36 hours of coolroom storage, is also reflected in the larger correlation values for temperature in the sensitivity analyses shown in Figs 4.24b and 4.25b. The total predicted growth from the model for the coolroom storage and distribution stage of Brie cheese is shown in Fig 4.28a, with up to a 2 log increase possible over the

4 day duration of this stage. The most likely outcome is a 2-3 generation increase (50^{th} percentile = log 0.74, 95^{th} percentile = log 1.3). Unsurprisingly, temperature is the most significant controlling factor for this stage (c = 0.90) (Fig 4.28b). It takes up to 36 hours for the product to reach 5°C after being taken from the wrapping room. This is reflected in the amount of growth predicted, as shown in Fig 4.24a. If this time could be reduced, a corresponding reduction in the potential *L. monocytogenes* growth could also be achieved

4.2.2.7 Brie Shelf life

It can be seen from Fig 4.29 that at 5°C the surface pH was ~7 at the commencement of the shelf life, and rose to ~8.5 at times during the duration of the shelf life. The data was not well represented by a normal distribution, but it was considered that this may have been due to localised changes in pH affecting the results. The a_w (and calculated salt concentration) of the cheese was much more variable (Fig 4.30), but the average a_w increased (corresponding to a decrease in calculated salt concentration) during the shelf life. The pH and salt concentration development at 10°C were similar in pattern to those observed at 5°C (Figs 4.32 and 4.33). The data were described well by a normal distribution for both parameters (fitting parameters are shown in Appendix E).

The shelf life growth predictions were based on a mean constant temperature, with a standard deviation of ± 0.5°C. The predicted outcomes of shelf life storage at 5°C are shown in Fig 4.31a, with the theoretical maximum growth possible up to of 36.6 logs (50^{th} percentile = log 24.3, 95^{th} percentile = log 29.3). The sensitivity analysis (Fig. 4.31b) shows the dominant controlling factor to be temperature (c = 0.97), followed by salt concentration (c = -0.20) and pH having little influence on growth, as it is well within the optimum range for growth. The predicted *L. monocytogenes* growth was evenly distributed over the span of the shelf life. Results for each 5 day period in the shelf life are presented in Appendix E.

The predicted *L. monocytogenes* growth during storage at 10°C is shown in Fig 4.34a, with a significant difference in growth from that predicted from storage at 5°C. The maximum theoretical predicted growth was 126 logs (50^{th} percentile = log 66.4, 95^{th} percentile = log 79.6), with the minimum predicted growth (log 46.1) exceeding the maximum for the 5°C trial.

Figure 4.29 - Mean pH during shelf life at 5°C (—),upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software

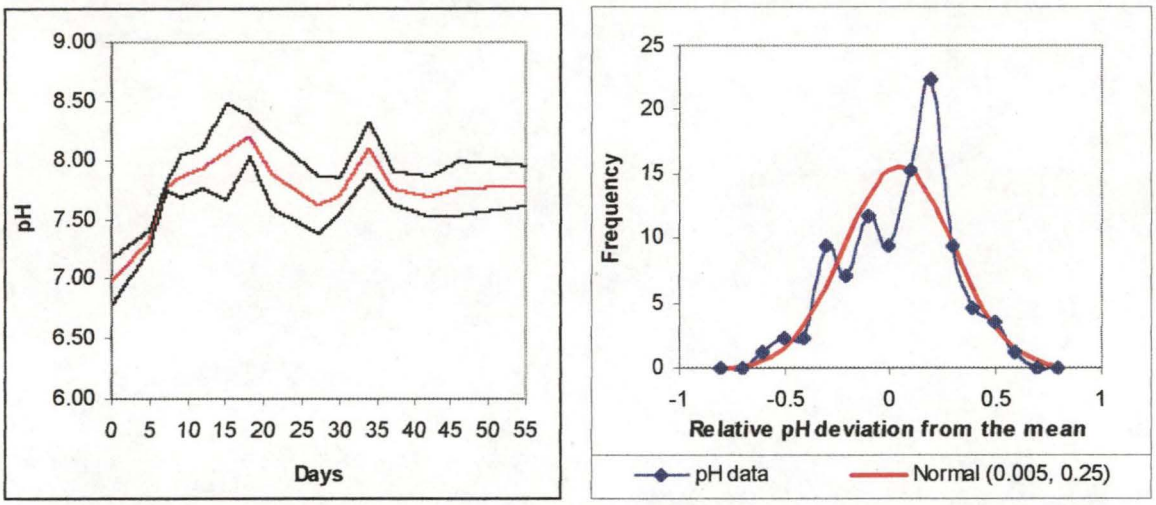


Figure 4.30 - Mean salt concentration during shelf life at 5°C (—),upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software

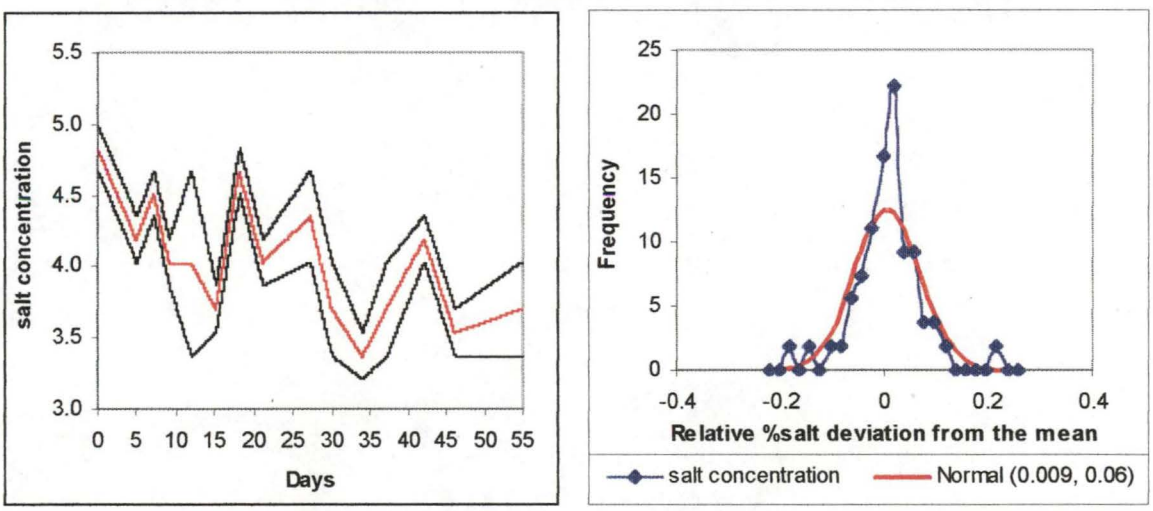


Figure 4.31 - Probability of potential *L. monocytogenes* growth and analysis of sensitivity to input variables during Brie shelf life at 5°C

4.31a **4.31b**

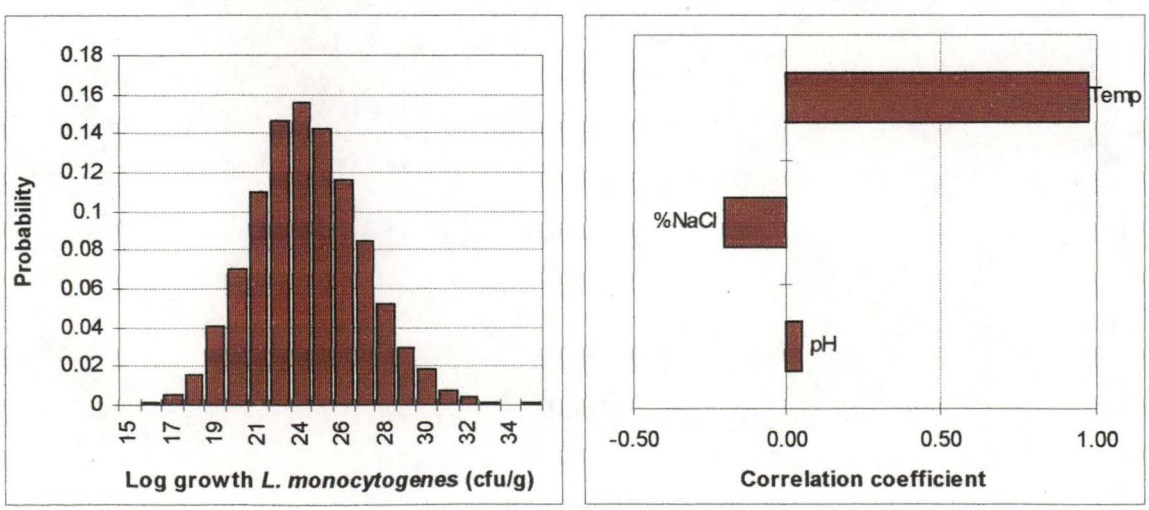


Figure 4.32 - Mean pH during shelf life at 10°C (—), upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software

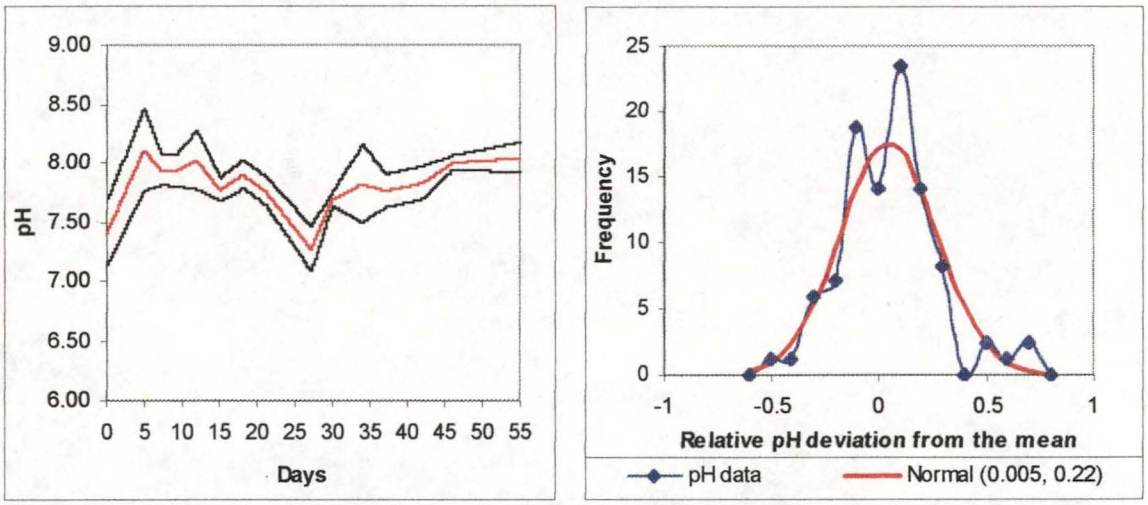


Figure 4.33 - Mean salt concentration during shelf life at 10°C (—), with upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software

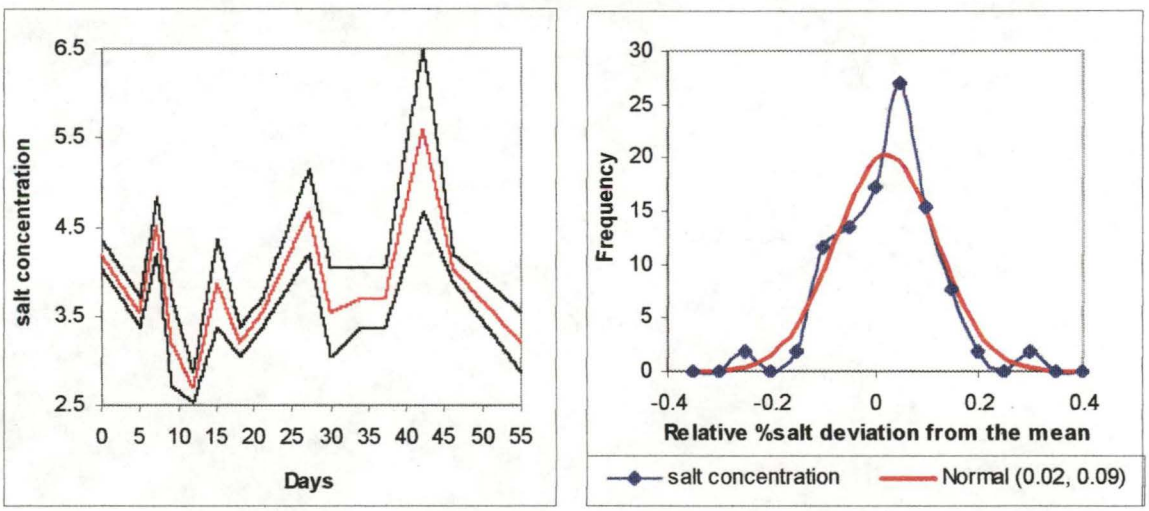
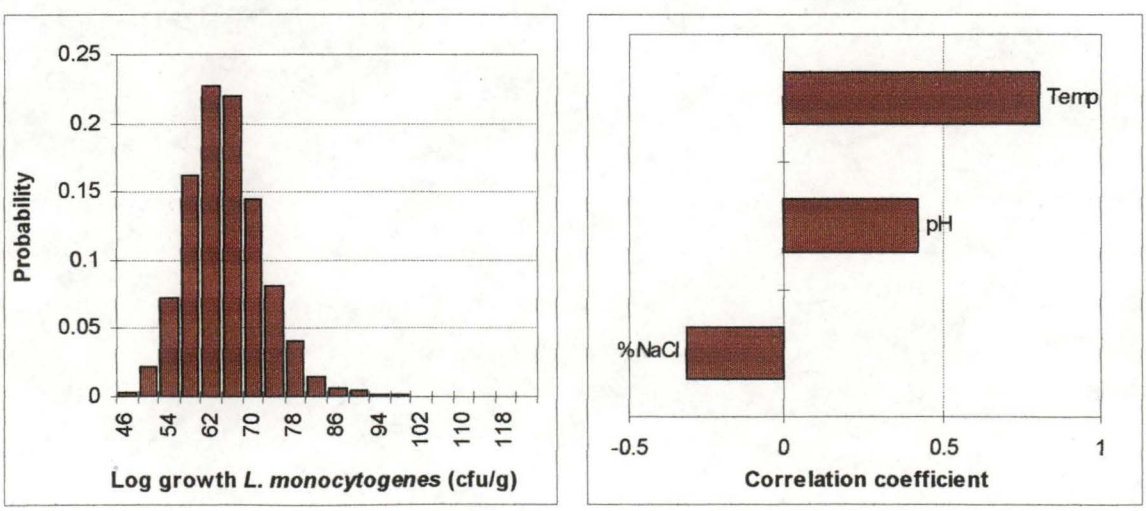


Figure 4.34 - Probability of potential *L. monocytogenes* growth and analysis of sensitivity to input variables during Brie shelf life at 10°C

4.34a **4.34b**



It can be seen from the sensitivity analysis (Fig 4.34b), that other factors begin to influence the growth of *L. monocytogenes* as the temperature becomes more favourable. These results demonstrated the necessity to specify an upper limit on model growth predictions to avoid the generation of non-sensical outcomes in the next stage of developing a detailed risk assessment. Despite previous observation that *L. monocytogenes* is a reasonably poor competitor (Grau and Vanderlinde, 1992), there was no inhibition apparent from the challenge tests detailed in Chapter 2. This led to the assumption that a maximum population density of 10^8cfu/g was possible for *L. monocytogenes* in this product, and the stochastic model adjusted to reflect this.

Figure 4.35 - Microbiological profile of 1kg Brie during shelf life storage at 5°C

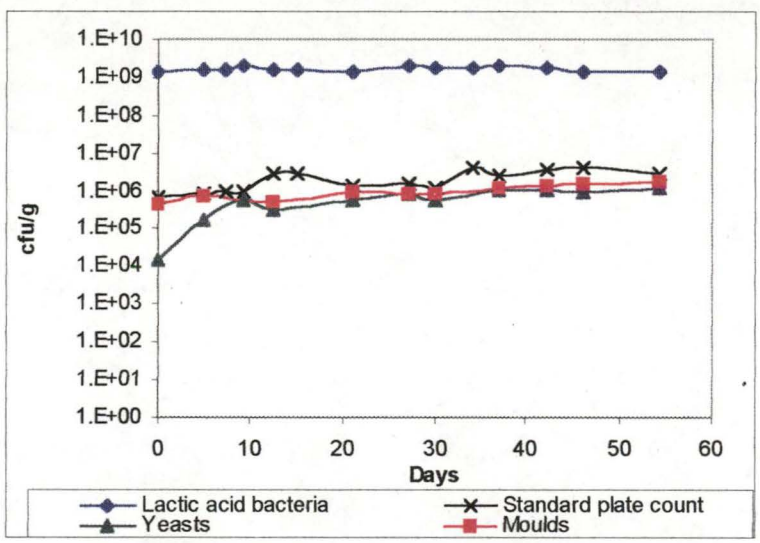
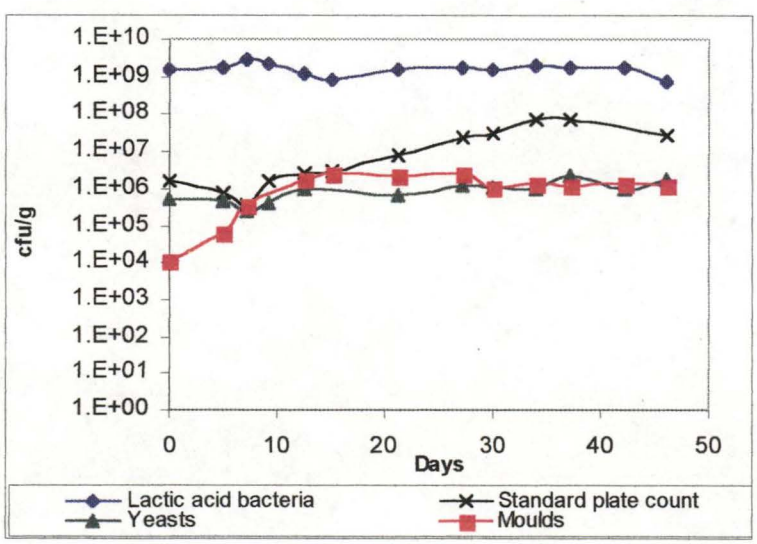


Figure 4.36 - Microbiological profile of 1kg Brie during shelf life storage at 10°C



Microbiological profiling of the product at 5°C (Fig 4.35) and 10°C (Fig 4.36) showed that the number of starter bacteria remained constant for the duration of the shelf life, at a level around 10^9cfu/g. At 5°C, all of the microorganisms enumerated remain relatively constant for the entire shelf life. At 10°C the standard plate count was observed to rise gradually, showing up to a 2 log increase, before levelling out at 10^7cfu/g. The yeasts and mould counts were consistent for each storage temperature, having maximum populations at 10^6cfu/g. This demonstrates the typical microbiological population of the cheese, with high levels of competing microorganisms.

The next stage of the assessment process involved refinement of the model predictions through the addition of parameters, developing a detailed risk assessment to quantify the likelihood of listeriosis as a result of consuming Factory 'A' Brie cheese, ie. to undertake a risk characterisation.

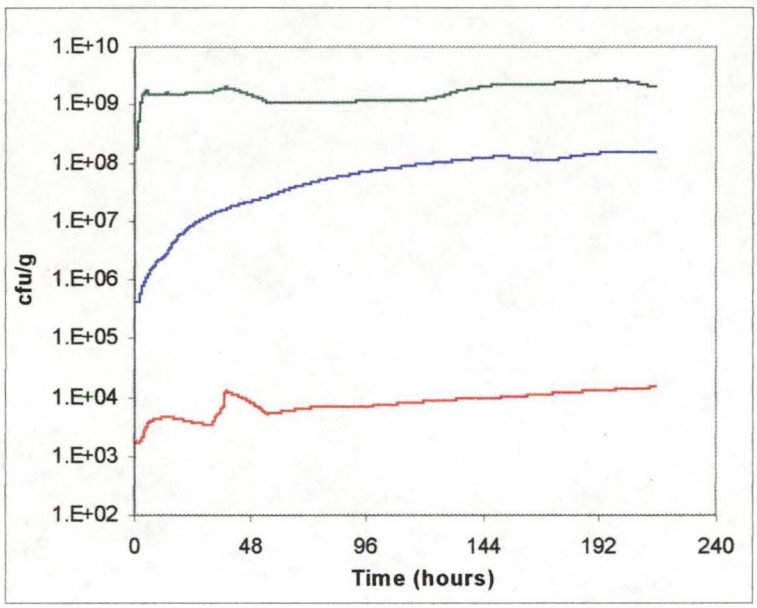
4.3.3 Brie detailed Quantitative risk assessment

4.3.3.1 Time of contamination

The microbiological profile of the Brie manufacturing process is shown in Fig 4.37. Numbers of starter bacteria were constant throughout the process, only varying when added into the fill tank during the morning's production and spraying after about 36 hours. Some spores lost their viability after addition by spraying, but numbers gradually increased to a level where the mould growth was visible on the surface of the cheese prior to wrapping. Total plate count rose slightly after the brining process, confirming the possibility that the brine may be a source of contaminants. However, Seilor & Busse (1990) studied the brine microbiota, finding it to be populated predominantly by halo-tolerant yeasts (levels up to 10^5cfu/mL).

Cheese total plate count numbers steadily increased to level of around 10^8cfu/g at wrap. For simplicity in the quantitative risk assessment (and lack of evidence to the contrary), the assumption was made that there would be an equal chance of *L. monocytogenes* contamination at any time during the entire manufacturing process, prior to wrapping of the cheese. Therefore a uniform distribution was established (Table 4.1) where time of contamination ranged from zero hours (modelling survival of cells from pasteurisation) to 216 hours (modelling contamination from manual handling during the wrapping process).

Figure 4.37 - Typical microbiological profile of Brie manufacture, showing levels of Starter bacteria (—), Standard plate count (—) and Moulds (—)



4.3.3.2 Level of L. monocytogenes *at end of storage and distribution*

The initial outcome predicted from the detailed risk assessment model was the level of *L. monocytogenes* at the end of the storage and distribution stage. The amount of predicted *L. monocytogenes* growth to this stage varies considerably (Fig 4.38), from no growth to the maximum growth level permitted by the model (10^8 cfu/g). The mean predicted level was log 3.01 (50^{th} percentile = log 2.93, 95^{th} percentile = log 7.42). The sensitivity analysis (Fig 4.39) allows observation of the factors most strongly correlating to the model outcomes: contamination time (c = -0.696); contamination level (c = 0.472); and lag phase (c = -0.34). The time when contamination occurs is shown to be strongly correlated with the amount of growth, an obvious outcome, since if the contamination occurs earlier in the process, then the organism has a longer time to multiply to higher levels. Therefore, if the assumption is incorrect that contamination has an equal chance of occurring throughout the process, this will have a large effect on the modelled outcome.

The importance of the Brie maturation phase is also highlighted, with maturation parameters ranked 4^{th}, 5^{th} and 6^{th}. The 7 day length of the maturation phase allows it to exert a significant influence on potential *L. monocytogenes* growth, as was shown in Section 4.6. It can be inferred from the sensitivity analysis that outputs where little

growth was predicted were due to simulations fulfilling the following conditions: contamination occurring late in the process; the level of contamination was low; and a lengthy lag phase was induced.

Figure 4.38 – Predicted level of *L. monocytogenes* at end of storage and distribution stage for contaminated Brie cheese

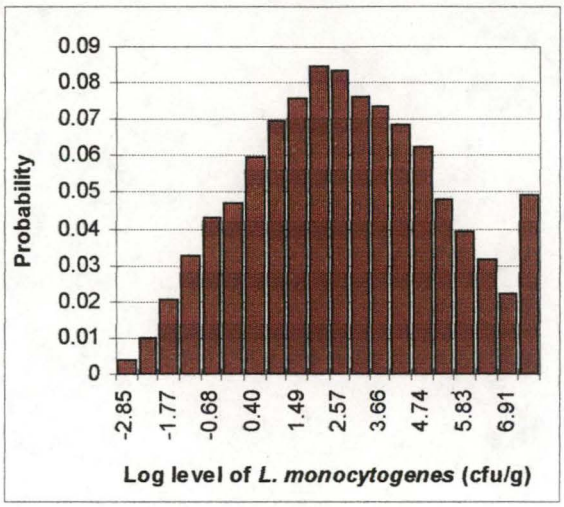
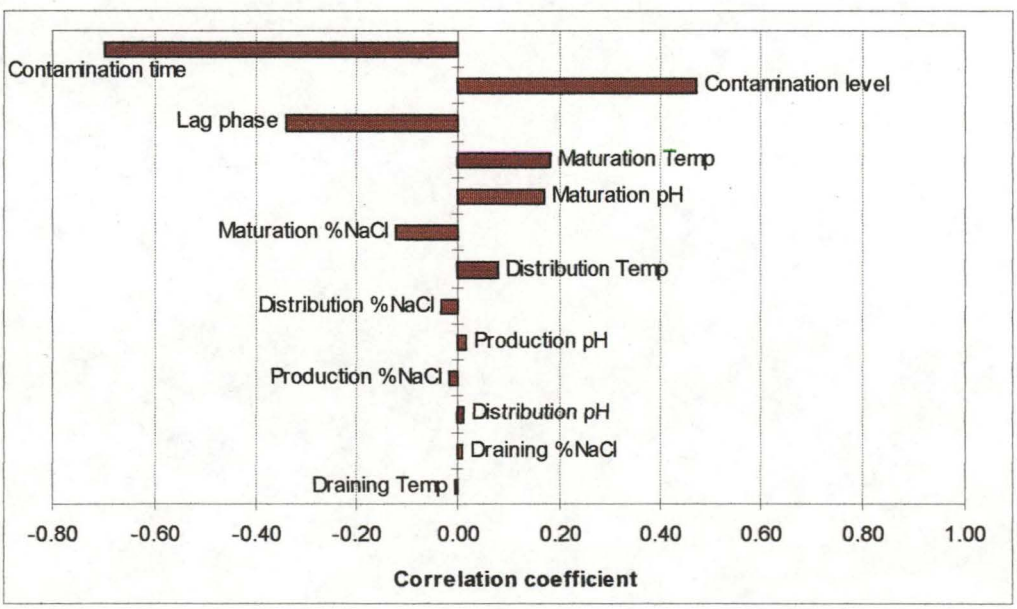


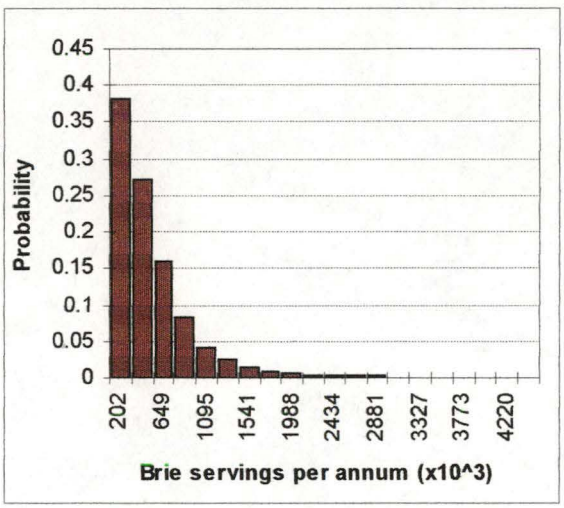
Figure 4.39 – Sensitivity analysis of model inputs for predicted level of *L. monocytogenes* in Brie cheese at end of storage and distribution



4.3.3.3 Number of serves

The number of serves *per annum* was calculated from Factory production records, divided by the serving size. Therefore iterations which included a large serving size, had a proportionally smaller number of serves *per annum*. The assumption was made that all cheese manufactured was consumed, and this led to be a mean value of 636,688 servings per year in Tasmania (Fig 4.40) and 2,522,697 in the rest of Australia.

Figure 4.40 – Number of Brie servings per annum in state of Tasmania



This is comparable on a *per capita* basis with estimates from US data (USDA, 2001) of 480,000 servings for a population the size of Tasmania. The calculated number of servings for the population of the rest of Australia based on the same US data is 17,000,000, however Factory 'A' only has an estimated 7% market share, which equates to 1,119,000 servings per year. This demonstrates that the calculated number of servings is similar in magnitude to previous survey-based estimates and is a useful tool in validating the consumption data used in generating model outcomes.

4.3.3.4 Level of L. monocytogenes *at time of consumption*

The next model output generated was the level of pathogen being presented to the consumer on the cheese at the point of consumption (Fig 4.41). It can be seen that on just under 70% of occasions when the cheese is contaminated with *L. monocytogenes*, it is capable of reaching the maximum population density specified in the model (10^8 cfu/g). Given the predicted levels at the end of storage and distribution, it appears the majority of this growth occurs during the product

shelf life. This demonstrates that the assumptions made for contamination level, one of the more poorly defined parameters in this risk assessment, are of little consequence in the final outcome.

Figure 4.41 – Predicted level of *L. monocytogenes* at time of consumption, for contaminated Brie cheese

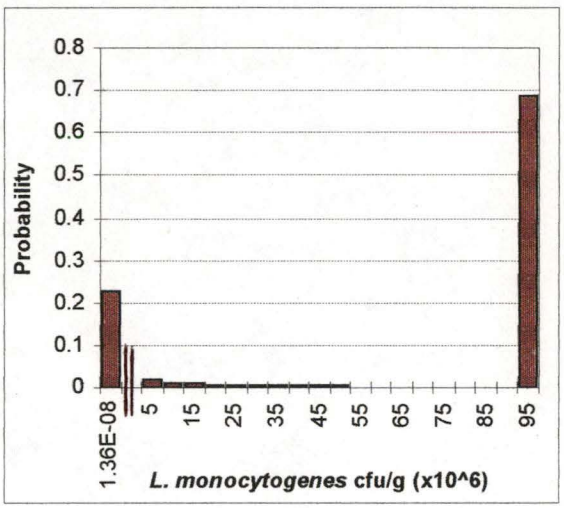
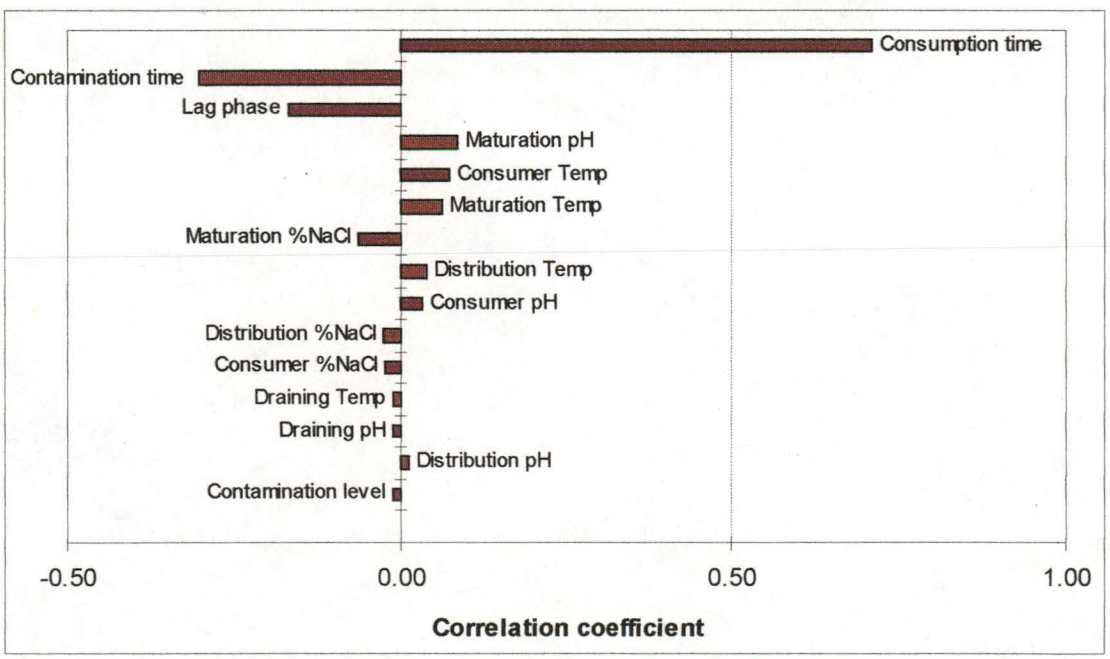


Figure 4.42 – Sensitivity analysis of model inputs for predicted level of *L. monocytogenes* in Brie cheese at time of consumption

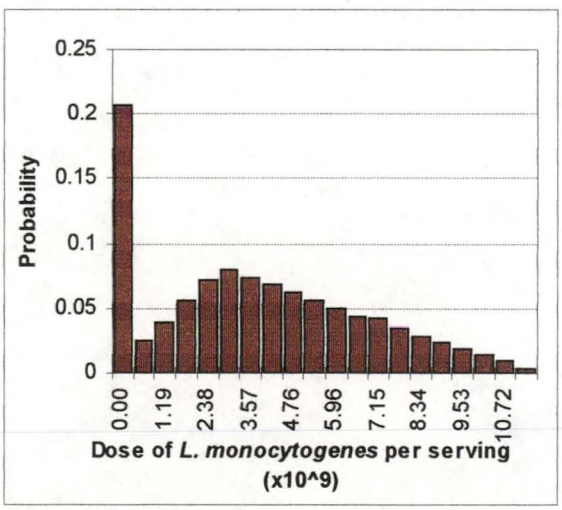


The initial contamination level was found to be of little significance for this output, since even very low levels of contamination were predicted to lead to high numbers of *L. monocytogenes* in the final product due to the favourable conditions in the cheese during the shelf life. This demonstrates that the assumptions made for contamination level, one of the more poorly defined parameters in this risk assessment, are of little consequence in the final outcome.

4.3.3.5 Dose of L. monocytogenes

Following from the level of *L. monocytogenes* on the cheese at the time of consumption, Fig 4.43 shows the predicted dose of *L. monocytogenes* presented to the consumer, with the mean level predicted at 3.81×10^9 cells (50^{th} percentile = 3.48×10^9, 95^{th} percentile = 9.61×10^9). As shown in Chapter 2, Dose was calculated by multiplying the level of *L. monocytogenes* (cfu/g) by the serving size of the cheese (g). The sensitivity analysis (not shown) for Dose is almost identical to Fig 4.42, except the direct correlation between dose and serving size is reflected by its large correlation value (c = 0.52).

Figure 4.43 - Predicted dose of *L. monocytogenes* on a serving of cheese.



4.3.3.6 Probability of infection per meal

In the calculation of listeriosis probability, the R–values proposed by Ross (*unpublished*) and Buchanan *et al.* (1997a) were compared. The R-value of Buchanan *et al.* (1997a) was discounted as it presented results which were not consistent with the reported number of cases. Using the Buchanan *et al.* (1997a) value of $R = 1.179^{-10}$, the number of listeriosis cases predicted in Tasmania was ~4,500 per annum in the susceptible population alone (results in Appendix E).

When this is compared to the number of reported cases per annum, an average of one per year, the Buchanan *et al.* (1997a) equation appears to over-estimate the risk by some 10,000 fold. Given this apparent inability of the Buchanan *et al.* (1997a) equation to accurately predict the risk, all predictions for number of listeriosis cases were based on the R-value proposed by Ross (*unpublished*) of $R = 1.87^{-14}$. Using this R-value, the probability of infection per meal estimated from the consumption of Factory 'A' 1 kg Brie was calculated according to Eqn 2.2. The risk ranged per meal from $1.35^{-14} - 2.32^{-4}$ (mean value = 7.17^{-5}). This value was generated for the cheese contaminated with *L. monocytogenes*, and did not account for the proportion of cheese containing no *L. monocytogenes*.

4.3.3.7 Predicted number of listeriosis cases per annum

The ultimate aim of the risk assessment process is to generate a health-based outcome. The final outcome for this study was a prediction for listeriosis cases per annum resulting from the consumption of Factory 'A' 1 kg Brie. Outcomes of listeriosis cases were derived for both the general population, and the susceptible population (as defined in Chapter 2). Using the Triangular distribution (0, 0.003, 0.043), ie an upper limit of 4.3% of cheeses contaminated for the year, the total predicted number of cases predicted by the stochastic modelling process to occur in Tasmania is shown in Fig 4.44. Combining the maximum predicted number from both populations results in a prediction of ~5.5 cases of listeriosis resulting from the consumption of Factory 'A' cheese in a year. The maximum output generated by the model for the rest of Australia predicts a worst-case of ~22 listeriosis cases per annum. However, the likelihood of this outcome is less than 1 in 100. The 50^{th} percentile output predicts a total of one listeriosis case per annum in the state of Tasmania, and 4.2 cases in the rest of Australia as a result of consuming Factory 'A' Brie cheese.

The predicted number of cases within each population group is shown in Tables 4.6 and 4.7, based on the relative susceptibilities and population estimates presented in Chapter 2. The distribution of outcomes and correlations calculated for the population of the rest of Australia are identical in shape to that presented for the state of Tasmania (as the inputs for the risk calculation are identical), but the predictions are adjusted for population and consumption trends as specified previously. A full description of all results is presented in Appendix E.

Figure 4.44 – Distribution of Listeriosis cases per annum in Tasmania as a result of Brie consumption a) general population; b) susceptible population Contamination frequency Triangular (0, 0.003, 0.043)

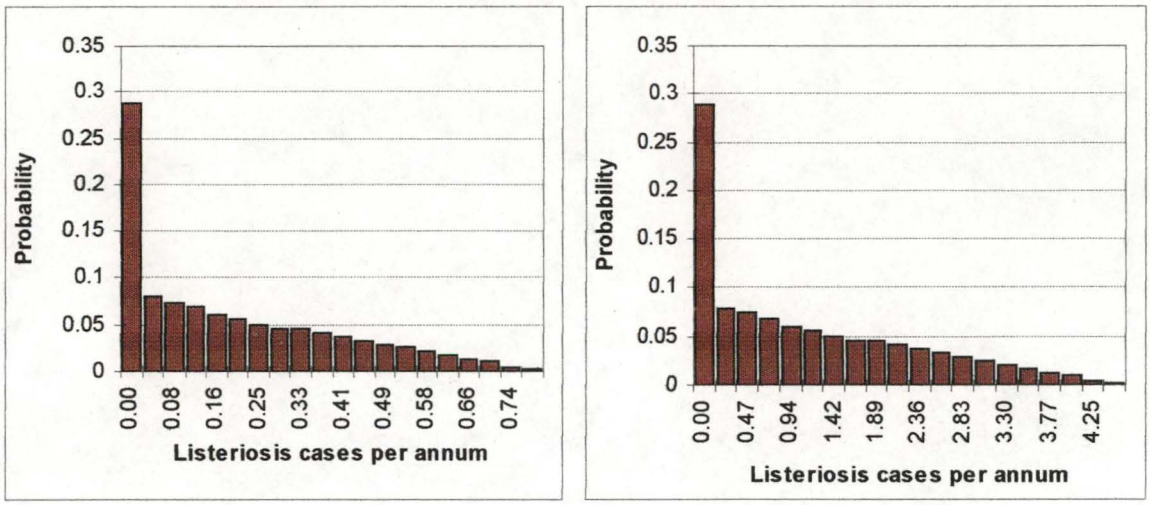
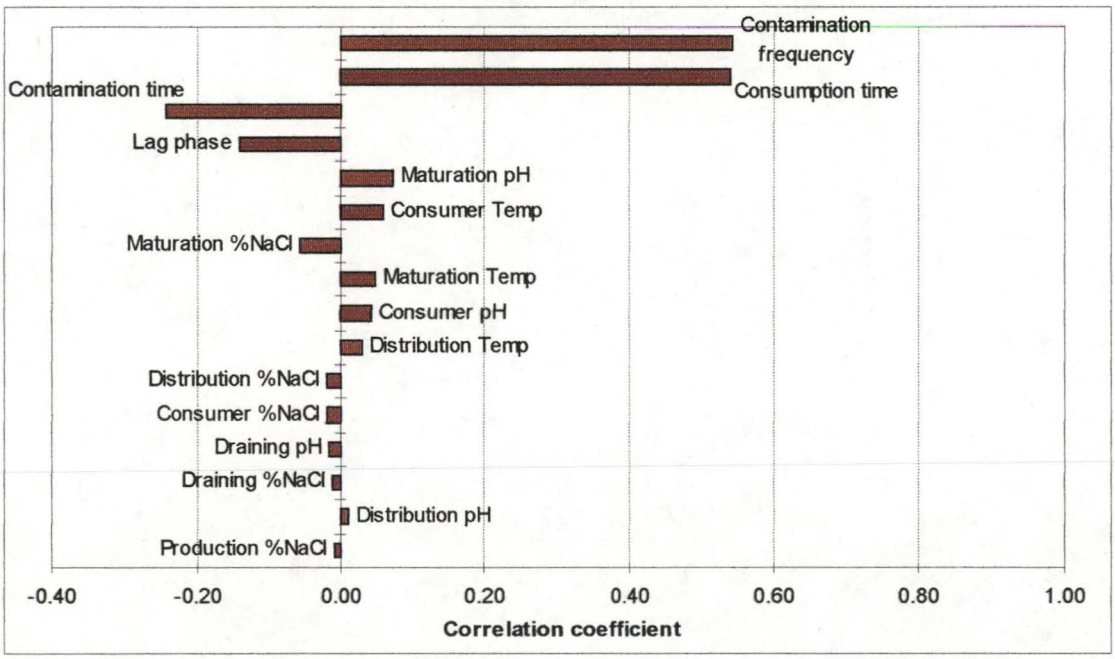


Figure 4.45 - Correlations for model inputs for risk of listeriosis per annum



It can be seen from Table 4.6 and 4.7, that although the susceptible population consists of ~18% of the population, almost six times as many cases can be attributed to the population sub-groups that are much more susceptible to listeriosis. Pregnant women are predicted to account for approximately one-quarter of the cases within the susceptible population. A large proportion of the cases are also attributed to diabetics, for whom the relative susceptibility was estimated as being the same as for the minimum within the susceptible group (x 28). This may be an over-estimation, and may be revised with further research. Within the 'normal population', the 30-39 year olds represent the highest risk group. While their estimated relative susceptibility is 0.79, because they consume more cheese than any other age class, they are predicted to account for over one-third of cases within the "normal population". Overall, the results generated in this risk assessment agree with observations of listeriosis cases occurring predominantly within susceptible population groups.

The sensitivity analysis (Fig 4.45) is almost identical to that presented for level of *L. monocytogenes* at time of contamination, however the addition of the contamination frequency results in it being equally correlated with consumption time (c = 0.54) for the most significant influence on the health outcomes. The sensitivity analysis also infers the influence of estimated parameter values on the modelling outcomes, with the four highest correlated inputs all originating from assumptions. The effect of parameter assumptions is tested in Section 4.3.3.6, by altering starting values, and the model adjusted to highlight possible risk mitigation strategies.

4.3.3.8 Alteration of input parameter estimates

To assess the validity of assumption made within the detailed risk assessment, parameter estimates were varied and the effect on model outcomes noted. This process also allows possible risk mitigation strategies to be evaluated for their effectiveness in limiting listeriosis.

Effect of consumer storage temperature

The effect of varying the storage temperature by 5°C was shown in Section 4.2.2.7, and the correlation of consumer storage temperature to the model outcomes was shown in Fig 4.45. A simulation was conducted to assess the effect of consumer storage temperature at 10 ± 0.5°C on model outcomes. The mean predicted level of *L. monocytogenes* at time of consumption increased to 9.44 x 10^7 cfu/g (10^{th} percentile = 10^8), with a resultant increase in the number of predicted listeriosis cases

per annum. A 50th percentile predicted number of 1.6 total cases in Tasmania and 6.6 cases in the rest of Australia, a greater than 30% increase in predicted cases upon the simulation including consumer storage at 5°C (Tables 4.6 and 4.7).

Table 4.6 – Predicted number of listeriosis cases per annum in Tasmania from consumption of Factory 'A' Brie, based on age / susceptibility

Tasmania					
	Minimum	**Mean**	**Maximum**	**50th percentile**	**95th percentile**
Cancer	0	0.051	0.198	0.038	0.147
Transplant	0	0.161	0.625	0.120	0.464
AIDS	0	0.127	0.494	0.095	0.367
Diabetes	0	0.264	1.028	0.198	0.764
Pregnant	0	0.341	1.324	0.255	0.984
Kidney	0	0.010	0.038	0.007	0.028
> 60 years	0	0.219	0.852	0.164	0.633
< 30 days	0	0.037	0.145	0.028	0.107
Susceptible population Total	**0**	**1.209**	**4.704**	**0.905**	**3.494**
1 – 9 years	0	0.007	0.027	0.005	0.020
10 – 19 years	0	0.008	0.032	0.006	0.024
20 – 29 years	0	0.026	0.102	0.020	0.076
30 – 39 years	0	0.074	0.288	0.055	0.214
40 – 49 years	0	0.039	0.153	0.029	0.114
50 – 59 years	0	0.056	0.218	0.042	0.162
General population total	**0**	**0.210**	**0.825**	**0.157**	**0.610**

Table 4.7 – Predicted number of listeriosis cases per annum in rest of Australia from consumption of Factory 'A' Brie, based on age / susceptibility

Rest of Australia					
	Minimum	**Mean**	**Maximum**	**50th percentile**	**95th percentile**
Cancer	0	0.202	0.785	0.151	0.583
Transplant	0	0.637	2.477	0.476	1.840
AIDS	0	0.503	1.958	0.376	1.455
Diabetes	0	1.048	4.074	0.783	3.026
Pregnant	0	1.349	5.248	1.009	3.898
Kidney	0	0.039	0.152	0.029	0.113
> 60 years	0	0.868	3.376	0.649	2.508
< 30 days	0	0.147	0.573	0.110	0.425
Susceptible popualtion Total	**0**	**4.793**	**18.643**	**3.583**	**13.848**
1 – 9 years	0	0.028	0.108	0.021	0.080
10 – 19 years	0	0.032	0.126	0.024	0.093
20 – 29 years	0	0.104	0.406	0.078	0.301
30 – 39 years	0	0.294	1.142	0.219	0.848
40 – 49 years	0	0.156	0.608	0.117	0.451
50 – 59 years	0	0.223	0.866	0.166	0.643
General population total	**0**	**0.837**	**3.256**	**0.625**	**2.426**

Effect of limiting shelf life

Given previous results in Section 4.2.2.7 which tended to suggest that the majority of *L. monocytogenes* growth occurs during the cheese shelf life, the effect of limiting the shelf life to 30 days on the predicted number of listeriosis cases was assessed. The contamination frequency used was as for the initial assessment (ie Triangular 0, 0.003, 0.043) and consumer storage was assumed to be 5 ± 0.5°C. The predicted level of *L. monocytogenes* at time of consumption dropped to 6.97×10^{7} cfu/g, and the corresponding probability of infection per meal ranged from 5.88×10^{-4} to 2.32×10^{-4}. The 50th percentile estimates of total listeriosis cases were 0.06 and 0.23 for Tasmania and the rest of Australia respectively, a reduction of 94% from the initial risk assessment results.

Effect of contamination frequency

The sensitivity analysis of listeriosis risk (Fig 4.45) indicates the largest determinant of risk to be the frequency of contaminated cheese, an intuitive outcome. The use of 4.3% contamination rate is thought to represent a worst-case scenario, due to the precautions which occur within the factory and the end-product testing that occurs. As stated in Section 4.2.4.1, the calculated frequency of contamination based on factory records was 0.3% (three contaminated batches per thousand). A simulation was generated assuming this contamination frequency to be the upper limit, with 0% as the average situation (Triangular 0, 0, 0.003). This simulation resulted in a 50th percentile prediction of 0.10 cases per annum in Tasmania and 0.25 cases per annum in the rest of Australia, a total of 0.35 cases (full results in Appendix E).

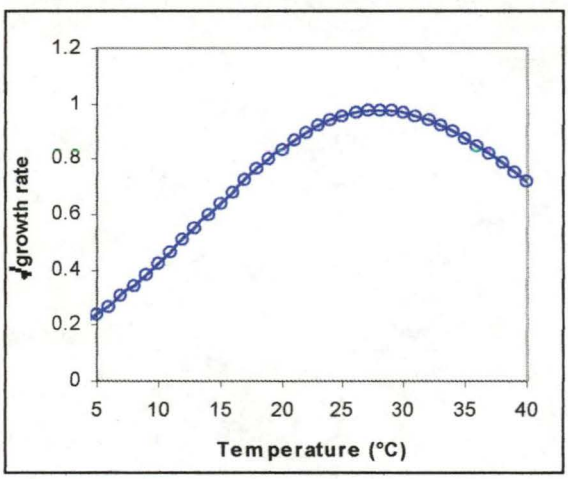
4.4 Discussion

Due to the relative novelty of the risk assessment process, there have been few attempts previously to quantify the risk of listeriosis from the consumption of surface-ripened cheese. Most published studies, apart from that of Bemrah *et al* (1998), differ from this thesis, in that they have been based on the consumption of a generic food group such as "soft cheese", with no quantification of the risk associated with a specific cheese type, let alone a specific cheese brand.

The initial Process Risk Model served the purpose of highlighting points within the manufacturing process which could be used to limit the proliferation of *L. monocytogenes*. Dissection of the sensitivity analyses for each step also showed which factors controlled *L. monocytogenes* growth. An unexpected model outcome

occurred from the initial portion of the production phase, where there was a strong negative correlation for temperature. The milk exits the heat exchanger at approximately 39°C (Table 4.2), above the optimum for growth of *L. monocytogenes*. McMeekin *et al* (1993) showed that a sharp decrease in growth rate occurs at temperatures above the optimum. This phenomenon is described in a square root plot of the Murphy-model predictions (Fig 4.46), which shows the growth rate of *L. monocytogenes* at 40°C to be similar to that at 15°C. This presents a strategy for limiting potential *L. monocytogenes* growth, as a further increase in the temperature of the milk leaving the heat exchanger will further limit the potential growth of *L. monocytogenes*. However, it must be ensured that increasing the temperature of the milk would not interfere with the growth of the inoculated starter bacteria.

Figure 4.46 – Murphy-model predictions growth rate over temperature range for milk (pH 6.7, %NaCl 0.7)



Acid development within the cheese was predicted to be the most significant factor limiting *L. monocytogenes* proliferation during the middle part of production (hours 4 – 12). Therefore it is important to ensure that proper and rapid pH development occurs. According to the classical definition of a Critical Control Point, however, acidification could not be classified as a CCP as it does not actually decrease or eliminate the hazard. The risk assessment clearly demonstrates that the most hazardous time, i.e. when most extensive *L. monocytogenes* growth is predicted to occur, is during the initial part of the production process prior to the development

of acidity to inhibitory levels. At this time the milk and curd temperature are in the optimum range for *L. monocytogenes* growth.

The PRM also served to highlight the importance of selecting appropriate time intervals to examine outputs, as the parameter correlations presented for the total 24 hr production period differed markedly to the outcomes presented from each 4 hr interval. The pH effect could be discerned in discrete four hour intervals: as the curd acidity developed to a point which began to inhibit *L. monocytogenes* growth, the correlation with pH became stronger. However, the correlation value for temperature over the 24 hr, was not as large as was inferred from inspection of the sensitivity analysis for each four hour interval. This was thought to be due to the early negative correlation (first 8 hours) and later positive correlations (hours 8-24) tending to cancel one another in the overall summary. This leads to an underestimation of the significance of temperature to the final model outcomes.

Also in the summary of production outcomes, salt concentration was found to be the second most significant parameter. However, it was shown through the 4 hour intervals that salt concentration did not become significant until brining, which occurs at the end of the production stage. Microbiological analysis of the brine has been designated as a Critical Control Point (Table 4.4). It is conducted on a weekly basis at Factory 'A' to ensure that the brine quality is maintained, and the risk of contamination is minimal. Due to the halotolerance of *L. monocytogenes*, should the brine solution become contaminated, the organism will survive and the brine may therefore serve as a potential reservoir of contamination. Brine may become contaminated through direct/indirect contact with the cheese factory environment, including condensation from walls and ceilings, as well as actual shedding of *L. monocytogenes* into the brine solution from contaminated cheese. Ryser & Marth (1999) listed at least one cheese recall traced to brine contaminated with *L. monocytogenes*. Increasing the salt concentration of the cheese will further limit the potential growth of *L. monocytogenes*, but any alterations to the salt content must be within the range that will maintain product quality and still make the final product acceptable to the consumer.

During the maturation process it was shown that the pH and a_w on the exterior of the cheese become very favourable for *L. monocytogenes* growth. Through the seconds and third day of maturation, it was shown that calculated salt

concentration was the most significant factor controlling growth. This is due to the variability in a_w, caused largely by absorption of moisture onto the cheese surface from the humid air in the ripening room, along with chemical reactions from the mould growth commencing the breakdown of lactic acid. Turning of the cheese late on Day 3 lessens the variability of a_w, as the cheese surface which had previously been on the underside of the shelving is now fully exposed to the humid air. Until this point, the chemical reactions on the underside of the cheese had not been able to proceed as fast as on the surfaces exposed to the air, and as a result the a_w has not become as variable on this surface.

All parameters were shown to have similar correlation values to the model outcomes of the maturation phase. However, the only viable control point available to limit the potential *L. monocytogenes* growth is to decrease the ripening temperature. The changes which occur in pH and a_w during this stage are part of the natural cheese maturation process, and there is little opportunity for risk mitigation strategies to be implemented. There are also obvious limitations in decreasing the maturation temperature, in that this may have the undesirable effect of inhibiting mould development on the cheese and adversely affect the maturation process. Due to the definitive characteristics associated with Brie cheese, any changes made to the maturation parameters to control the potential growth of *L. monocytogenes* have to also be carefully considered in terms of product quality.

The outputs generated in the Process Risk Model have shown that all three variables of temperature, pH and a_w are significant at some stage during Brie production. The analysis also identified points within the manufacturing process as contributing to the growth of *L. monocytogenes*, and several control points were identified which were suggested as methods for limiting the proliferation of *L. monocytogenes*. However, the outcomes generated in the detailed risk assessment graphically demonstrate that should contamination with *L. monocytogenes* occur, the organism is capable of reaching high levels in the cheese, almost regardless of the measures taken within the factory. Sulzer *et al* (1992) studied the development of *L. monocytogenes* in Camembert cheese by inoculation into the cheese milk, or by addition at very low levels (0.5-2.5 cfu/cm^2) during ripening. The authors found that regardless of when contamination occurred, levels of up to 10^4-10^6cfu/cm^2 were reached after normal ripening of 2-3 weeks. The same authors, (Sulzer & Busse, 1993), found that 2 weeks after production, the time when a consumer would first be

able to purchase the cheese, each viable *L. monocytogenes* cell which had contaminated the cheese surface would have been able to form a colony with approximately 10^4-10^6 cells. Wan *et al* (1997) also found *L. monocytogenes* able to reach levels up to ~ 10^7 cfu/g after 21 days in Camembert cheese.

The factory-designated Critical Control Points highlighted in Tables 4.3 & 4.4 were mainly aimed at preventing microbial contamination of the product, highlighting equipment and processes which directly contact the cheese, and therefore increase the potential for contamination. Processes such as the addition of ingredients, hooping, wrapping all have the potential to introduce contamination to the cheese, and as shown in the PRM outcomes, should contamination occur, it has the potential to grow to high levels. However, none of these form a true CCP as they cannot eliminate a hazard and these processes should be controlled through HACCP support programs of hygiene and sanitation and the employment of GMP. Process steps where some control can be exerted (ie Raw milk receival temperature, pH level during production and warehouse storage temperature) are important for controlling the extent of hazard proliferation. However, these steps can only be classed as Control Points given the levels which *L. monocytogenes* can potentially grow to during the shelf life of the product. Therefore, although it is acknowledged that all the highlighted steps in Tables 4.3 and 4.4 are essential for limiting the probability of contamination with *L. monocytogenes*, the only true CCP for the entire process is the pasteurisation process, with control points in place afterwards to prevent post-pasteurisation contamination.

For all predicted growth modelling, it was assumed that *L. monocytogenes* growth was unaffected by mould and starter culture numbers. The results presented in Chapter 3 and data from published reports (Ryser & Marth, 1987b; Genigeorgis *et al,* 1991b; Ryser & Marth, 1999) tend to suggest there is no inhibition of *Listeria monocytogenes* caused by the *Penicillium* mould on the surface of the cheese, or the lactic acid bacteria in the interior of the cheese. Ryser & Marth (1987b) inoculated the surface of 10 day-old Camembert with several strains of *L. monocytogenes,* to simulate contamination occurring in the ripening room. *L. monocytogenes* strain Scott A grew to levels of 4.9 x 10^4 cfu/g from an inoculation level of 30 cfu/g. These final levels were lower than achieved on cheese inoculated at an earlier stage of manufacture. Another study by the same authors suggested that factors associated with the surface mould may even have a beneficial effect on *L. monocytogenes*

growth, with Ryser & Marth (1988) showing enhanced growth of *Listeria* strains in filter-sterilised Camembert cheese whey previously cultured with *P. camemberti* than in uncultured whey over a pH range of 5.60 to 6.80.

A number of factors contribute to Brie sustaining high levels of *L. monocytogenes* growth. The manufacture time of Brie is 9 days, mostly at near ambient temperature (> 15°C), with the possibility of contamination occuring throughout this time. This extended process time, combined with the lengthy shelf life of the product results in sufficient time for almost any contaminating level of *L. monocytogenes* to grow to high levels in the finished product prior to consumption. Sulzer *et al* (1992) stated that *L. monocytogenes* development was critically affected by the pH of the cheese. At a rind pH of ≥ 5.5, *L. monocytogenes* counts increased 3-4 orders of magnitude within 1 week at the ripening temperature of 17°C, and 1-2 orders of magnitude at 5°C. It was also found that ripening and storage temperature influenced *L. monocytogenes* growth, with storage at 7°C after packing (8 days after production) yielding cell counts 1.5 times higher that storage at 4°C within 2 weeks of storage.

The results of the detailed risk assessment highlight the deficiencies of the initial Process Risk Model, and the need for refinement with additional parameters. The control points identified earlier may be effective in limiting initial *L. monocytogenes* growth, however the organism will grow to high levels during the course of storage and shelf life. The sensitivity analysis of the predicted number of listeriosis cases also demonstrates the significance of the assumptions made for determining risk assessment outcomes. The three most significant parameters highlighted in the sensitivity analysis were consumption time, contamination time and lag phase, which were all based on assumptions.

Given the reliance of the model on assumptions, the outcomes compare well with previous risk assessment studies. The study of Bemrah *et al* (1998) on raw milk soft cheese, estimated 57 cases of listeriosis per year in a population of 50 million (1.14 cases per million people). The difference between the current study and that of the Bemrah *et al* (1998) study, is that it did not account for any *L. monocytogenes* growth occurring during the manufacture phase. This resulted in Bemrah *et al* (1998) predicting a much lower level of *L. monocytogenes* in the final product than shown in this study, but a predicted contamination rate of 65%. If the same contamination rate

(Triangular 0, 0.32, 0.65) was applicable to the Brie cheese considered in this study, the mean number of listeriosis cases was predicted to be 29 in Tasmania and 116 in the rest of Australia (results in Appendix E). This total of 145 cases consitutes an average incidence of 7.6 cases per million inhabitants. This degree of incidence is similar to those reported in European countries where raw milk cheeses are widely consumed (see Chapter 1). Farber *et al.* (1996a) calculated the average probability of illness to be in the order of 2.5×10^{-6} to 2.5×10^{-4} per meal, based on Canadian consumption statistics which suggested the annual intake of soft cheese was approximately 5.5 kg, some 30-50 times higher than the consumption calculated for the Australian population. This is comparable with the mean estimate of risk per meal generated in this study of 9.9×10^{-5}.

Several risk mitigation strategies were predicted to limit the number of potential listeriosis cases. A reduction in the shelf life to 30 days saw a significant reduction in the predicted number of listeriosis cases. This strategy would obviously have a financial impact on the product, and given that the product currently constitutes a relatively low risk, may not be a viable option. The importance of maintaining the cold chain was shown, with a 30% increase in listeriosis cases with the cheese stored at 10°C, rather than 5°C during its shelf life. Use of the 0.3% contamination frequency showed that one case of listeriosis might be expected to be caused by the consumption of Factory 'A' Brie cheese every 3 years, or after the consumption of ~ 375 tonnes of cheese (~ 1.1 tonnes of which is contaminated with *L. monocytogenes*). Given an approximate mortality rate of 30% (Chapter 1), it might be expected that a death would occur once in ten years. This outcome may be more reflective of the 'real life' situation, but it still begs the question of whether this is an acceptable outcome.

4.4.1 Conclusions

The results presented here infer that in the vast majority of cases, when contamination of Brie with *L. monocytogenes* occurs, the organism can grow to levels capable of causing disease in susceptible consumers. Therefore, it is considered that control of *L. monocytogenes* in this product must lie in heat treatment of the milk through HTST pasteurisation, and the use of Good Manufacturing Practice to ensure post-pasteurisation contamination does not occur. The risk may also be reduced by strict control of temperature during maturation and consumer handling, but this is only likely to reduce the amount of growth possible,

not actually reduce the hazard. Therefore the only true Critical Control Point in this process is the initial heat treatment of the milk, with control of post pasteurisation contamination through hygienic practices within the factory environment.

The addition of data to develop the quantitative risk assessment demonstrates that the risk of contracting listeriosis from the consumption of Brie cheese is primarily dependent upon the percentage of cheese contaminated. The predicted number of cases per annum is within the order of magnitude observed in the number of reported cases in the state of Tasmania, and the rest of Australia, giving credibility to the assessment outcomes and the model describing the factors leading to listeriosis from Brie. Although, it must also be considered that there are many other potential sources of *L. monocytogenes* which contribute to the observed incidence. The importance of making accurate assumptions as realistic as possible for the risk assessment can be seen, in that parameters that were most significantly correlated with risk of human illness are those for which data was not available and input values had to be estimated.

5. PRODUCT CASE STUDY 2 – 1 KG RICOTTA

5.1 Introduction

Product Case Study 2 presents a quantitative risk assessment on the consumption of 1 kg Ricotta manufactured by Factory 'B'. It follows a similar sequence to the previous Case Study, with the cheesemaking process modelled in terms of temperature, pH and salt concentration, predictions for *L. monocytogenes* growth generated and the risk of listeriosis estimated.

5.1.1 Ricotta

Ricotta is a fresh, high-moisture cheese made from whey, often enriched with extra milk or cream. It has a mild sweetish flavour, a moist texture and the lactose imparts a 'sweet, snowy white flavour' (Rogers, 1995). Ricotta originated in Italy, and was initially a product of opportunism forced by poverty. It was traditionally made from the whey remaining after the production of cow's milk Mozzarella. However, consumer demand for a softer, creamier Ricotta cheese has meant it is now frequently made from whole milk.

The cheese can be consumed as a ready-to-eat product, served straight from the refrigerator as a dessert cheese with fresh fruits. However, it can also be used as a basis for cheesecakes, stuffings and fillings in baking. Ricotta made from whole milk can be used direct for eating and for cooking, resembling highly creamed cottage cheese. The popularity of Ricotta in Australia has become stronger in line with the trend towards healthy eating and reducing overall fat in the diet. Australian consumption has increased in the last several years, market share increasing from 1000 tonnes in 1992 to 2846 tonnes in 1997 (Willman, 1998). The first known isolation of *L. monocytogenes* in dairy products within Australia occurred from Ricotta (Venables, 1989). It has also been demonstrated that *L. monocytogenes* can readily grow on this cheese, with Ricotta and several other whey cheese varieties having been implicated as causes of listeriosis (see Chapter 1). The Ricotta studied in this project, from Factory 'B', was manufactured from a combination of whey and whole milk, with the final product having between 70-80% moisture and a pH of 6.2 - 6.4.

5.2 Materials and Methods

5.2.1 Characterisation of the Ricotta cheesemaking process

The entire Ricotta cheesemaking process was characterised within the factory, according to the methods outlined in Section 2.2, commencing from the point of whey receival through to packaging. Parameter distributions (temperature, pH and calculated salt concentration) were defined as outlined in the previous Case Study, commencing subsequent to the heat treatment, at the point where the curd temperature reached 40°C. Both 300g and 1kg sized Ricotta are manufactured at Factory 'B'. The 1kg size was selected for study because the cooling time is longer and it retains more moisture due to its size. Therefore, this product may offer conditions slightly more favourable for the proliferation of *L. monocytogenes*. The Ricotta manufacturing process was arbitrarily divided into stages for improved parameter definition. These stages were defined as Production (0-20 hrs), Storage and Transport (day 2) and Shelf Life (days 3-30). The production stage was subsequently subdivided into 4 hr segments, to more accurately account for rapidly changing parameter values.

5.2.2 Analysis of Ricotta final product attributes

Samples of final product were analysed 24 hrs after vacuum packaging, to characterise the distribution of pH values and calculated salt concentrations. A microbiological profile was conducted according to methods outlined in Chapter 2, with enumeration of yeasts, moulds and standard plate count.

Once the cheesemaking process and finished product had been characterised, the model was established to predict the risk of listeriosis from consumption of Factory 'B' Ricotta. Specific assumptions necessary for the Ricotta Case Study are outlined in the Section below.

5.2.3 Risk assessment - assumptions

As highlighted in the Case Study 1, a lack of quantitative data necessitates the adoption of several assumptions to predict a health outcome from a Quantitative Microbial Risk Assessment. Several of the assumptions already outlined in Chapter 2 were made specific for Factory 'B' Ricotta. These assumptions are listed in Table 5.1 and justified in the following sections.

Table 5.1 - Distribution inputs for Detailed Ricotta risk assessment

Variable	Description	Unit	Distribution / Model
Time of contamination	Time during manufacture when cheese is contaminated	hr	Uniform (0, 20)
Time of consumption	Time during shelf life when cheese is consumed	hr	Triangular (48, 144, 720)

5.2.3.1 Frequency of contamination

Factory records indicated there had been no recorded detection of *L. monocytogenes* in Ricotta samples, therefore estimates of contamination were based on literature data. Surveys conducted on Ricotta have shown that it is capable of sustaining high microbial loads. A 1981 Australian survey (NHMRC, 1983) found 37% of Ricotta cheeses had high numbers of *E. coli*, with levels up to 10^{6}-10^{7} cfu/g reported. Staphylococci were detected in 4%of samples with levels up to 10^{2}-10^{3} cfu/g. High numbers of yeasts (in 68% of samples) up to 10^{9} cfu/g and moulds were detected in 32% of sample units with levels as high as 10^{5}-10^{6} cfu/g.
L. monocytogenes was not monitored in this survey as it had not yet emerged as a well known pathogen. The pH range found in the survey of Ricotta samples was very wide, from 4.5 to 6.6, which may have indicated a lack of control in the cheesemaking process, or merely different manufacturing methods. During a survey of Australian factories and products, Venables (1989) recovered *L. monocytogenes* from several batches of Ricotta cheese.

Factory 'B' did not have many of the GMP systems in place that were mentioned in the previous Case Study for Factory 'A'. However, quarterly product sampling had failed to detect any *L. monocytogenes* since it had commenced (2 year period), and there had also been no *Listeria* detected in the monthly environmental sampling which had been conducted over the same timeframe. No positive isolations of *L. monocytogenes* were found in the limited sampling conducted for this study. Therefore, with no available data to estimate a frequency of contamination within the product, the same contamination frequency was assumed as for Case Study 1.

5.2.3.2 Time of contamination

To define an appropriate distribution for the time of contamination, a microbiological profile of the process was conducted to detect possible sources of contamination. Results (shown in Section 5.3.3.1) were used to validate the uniform distribution in Table 5.1. Levels of airborne contamination were estimated by exposing PCA and OGYE agar plates during the cooling phase of the Ricotta cheese to enumerate total viable aerobic count and yeasts and moulds respectively. The plates were placed beside the cooling cheese, thus gathering similar levels of microbial contamination that the cheese was exposed to. The plates were collected after 3 hrs exposure time, incubated at standard conditions and enumerated according to the protocols outlined in Section 2.2.3, and the rate of contamination calculated as cfu/cm^2/hr.

5.2.3.3 Time of consumption

The product is labelled with a 'Use by' date of 28 days from the date of manufacture. It was assumed that a large proportion of the cheese will be consumed within the first week of the shelf life, and that consumption of the product after the suggested use-by date would be minimal. It was assumed in model inputs (Table 5.1), that the product may be consumed up to 2 days after the 'Use by' date. Due to the product's susceptibility to spoilage, only intact packages would be organoleptically acceptable at this point, therefore limiting the probability of consumption after the 'Use-by' date. As in the previous Case Study, a Triangular distribution was used to describe the time of consumption (Table 5.1).

5.2.3.4 Exposure assessment

Production records from Factory 'B' were used to estimate the annual production of Ricotta cheese, all of which is consumed within the state of Tasmania. Ricotta production at Factory 'B' is relatively new, initiated as a method for utilising the leftover whey from Feta manufacture. The production volumes are therefore still quite low and dependent upon the demand for Feta. Approximately 4 tonnes of Ricotta is produced per year. Based on consumption statistics outlined in Chapter 2, the total calculated consumption of Ricotta in a population the size of Tasmania would be approximately 20 tonnes, equating to Factory 'B' having a market share of 20%. This factor was used in the final calculation of number of listeriosis cases due to consumption of Factory 'B' Ricotta.

5.3 Results

5.3.1 Characterisation of the Ricotta manufacture process

The Ricotta manufacture method used in Factory 'B' is a standard process for this variety of cheese. A typical manufacturing schedule is shown in Tables 5.2. Temperature, pH and calculated salt concentration profiles are shown in Figs 5.1a, 5.1b and 5.1c respectively.

Fresh whey remaining from Feta production is utilised, and stored for several hours at the production room ambient temperature (~ 28°C) prior to use. A typical starting whey volume is approximately 450 litres, to which 10% (v/v) raw milk is added, and approximately 0.1%(w/v) salt. The greater the quantity of whole milk added, the greater the final yield and the closer the product resembles ordinary cheese.

Table 5.2 - Typical manufacturing schedule and parameter values for 1kg Ricotta

Step	Time		Temp (°C)	pH	a_W
Whey receival	Day 1	7:00 AM	27.6 ± 2.7°C	6.27 ± 0.21	0.996 ± 0.002
Begin steam heating		8:25 AM	27.9 ± 2.3°C	6.26 ± 0.20	0.996 ± 0.002
Add salt		8:35 AM	32.7 ± 8.2°C	6.19 ± 0.15	0.997 ± 0.001
Add milk		9:05 AM	64.5 ± 10.6°C	6.00 ± 0.44	0.995 ± 0.001
Add acidulant		9:10 AM	72.3 ± 7.2°C	5.91 ± 0.56	0.995 ± 0.001
Stop heat		9:30 AM	80.6 ± 8.3°C	6.28 ± 0.11	0.996 ± 0.001
Scoop curd into moulds		9:50 AM	79.4 ± 11.0°C	6.24 ± 0.15	0.996 ± 0.001
Cover		10:30 AM	61.3 ± 8.3°C	6.22 ± 0.16	0.995 ± 0.001
Move into coolroom		4:30 PM	11.8 ± 4.5°C	6.24 ± 0.18	0.995 ± 0.001
Vacuum-packed	Day 2	7:45 AM	3.3 ± 1.6°C	6.26 ± 0.16	0.996 ± 0.001
Heat shrink		8:30 AM	98°C ± 3.6°C	6.26 ± 0.16	0.996 ± 0.001
Move into coolroom		9:00 AM	7.8 ± 4.5°C	6.24 ± 0.18	0.995 ± 0.001

Figure 5.1a - Mean temperature of Ricotta manufacture (—) with upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software

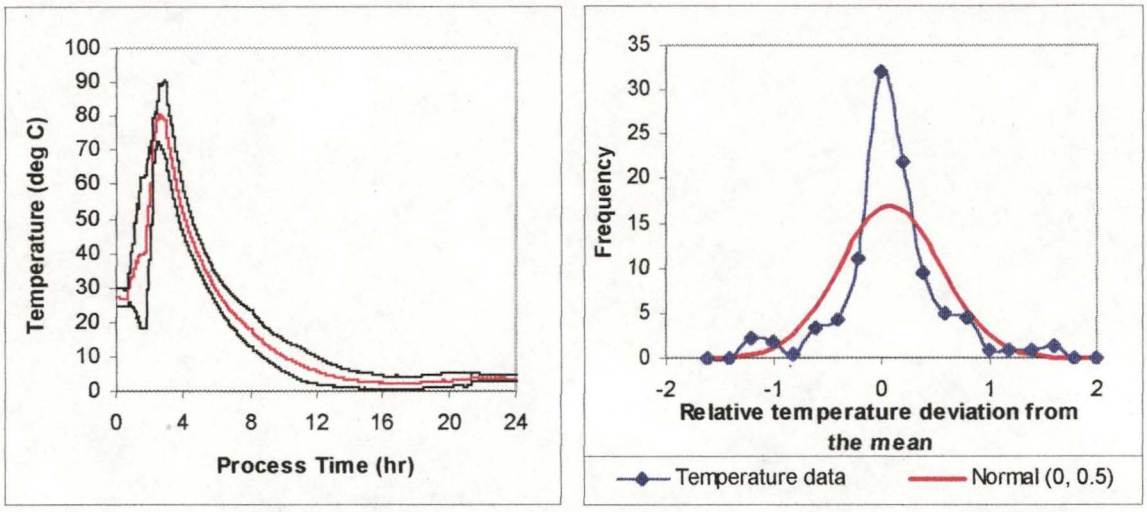


Figure 5.1b - Mean pH of Ricotta manufacture (—) with upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software

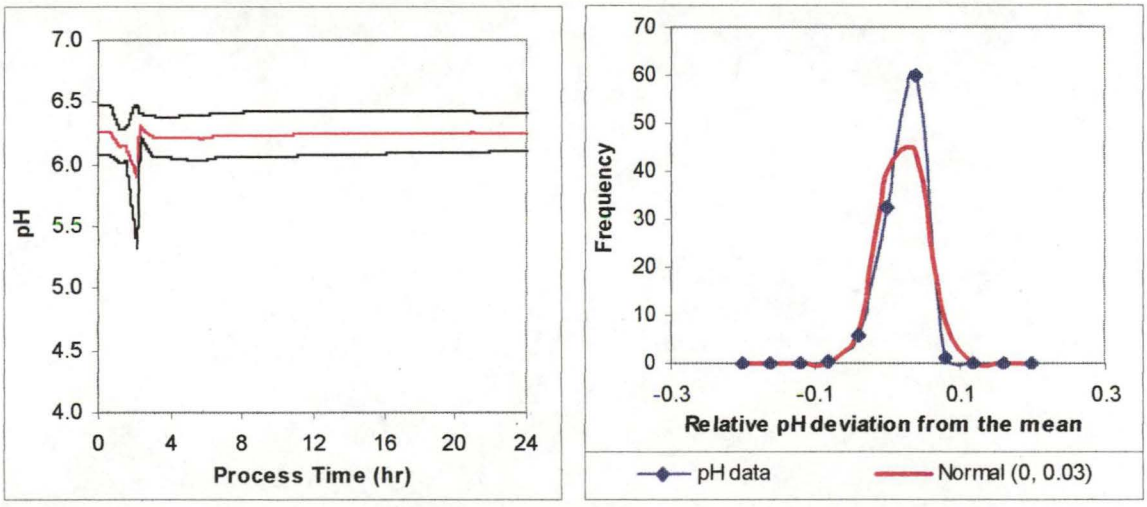
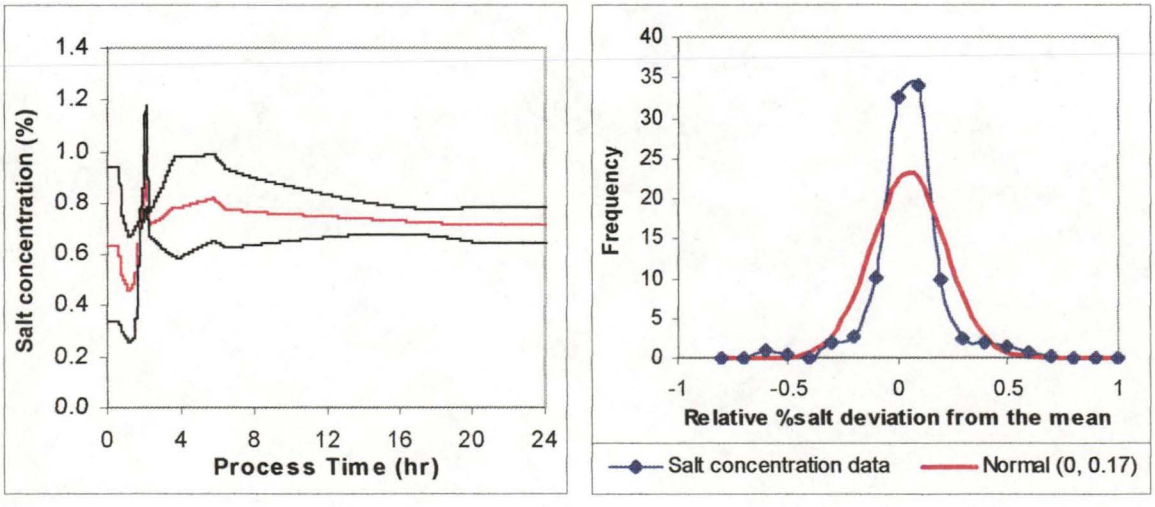


Figure 5.1c - Calculated salt concentration of Ricotta manufacture (—) with upper and lower limits (—) and Comparison with Normal distribution from Bestfit software



Direct infusion of steam into the whey mass heats it to 83-88°C after a short time, and the milk and acidulant are added during the heating process. The whey is heated at a rate of ~ 5°C per minute, and the maximum temperature is maintained for 25 to 30 minutes. Once the acidulant is added, the steam is used briefly (< 60 secs) to agitate the mixture to achieve an even spread of the acidulant throughout the whey mass. The steam agitation is removed and the combination of high temperature and lowered pH results in the denaturation and coagulation of the whey proteins, the mechanism of which was described by Galani and Apenten (1996).

The resulting curd entraps air with the small amount (0.25%) of fat in the whey, upon precipitation and coalescing, floating to the surface. Proper flotation of the curd depends on whether an optimum pH is attained, and on minimising agitation of the curd mass (Davis ,1976). The coagulated curd is allowed to stand for a short time and then skimmed off the surface, placed into perforated baskets and allowed to drain without pressing for 3 to 5 hours at room temperature.

5.3.1.1 Ricotta Ingredients

Ingredients used in Ricotta manufacture are whey, raw milk, salt and acidulant. There is very little risk presented by any of the ingredients, as they are all added prior to, or during, the injection of steam. The risk of contamination from Feta whey was shown to be minimal by Papageorgiou & Marth (1989), who found only 3.2% of the *L. monocytogenes* cells initially present in the milk were lost in the whey during Feta manufacture. Therefore, should the original Feta cheese be grossly contaminated with *L. monocytogenes*, the resultant numbers present in the whey for Ricotta production would be easily inactivated by the cooking process (see Section 1.5.2.2). Papageorgiou *et al.* (1996) stated that whey contaminated with very high levels of *L. monocytogenes* would be suitable for whey cheese manufacture, as the bacteria would not survive processing. The severity of the cooking process indicates whey handling need not be classified as a CCP.

Raw milk is stored at 4°C prior to addition to the whey as it is being heated, but as the time and temperatures used are well in excess of the minimum batch pasteurisation requirements (ANZFA, 1999), there is very little risk of *L. monocytogenes* surviving the cooking process. The risk presented through the use of raw milk is the close proximity of the raw milk receival area to the processing area, as there is no physical separation within Factory 'B', leading to an increased risk of post-pasteurisation contamination. Steps were being taken at the time of writing to

rectify the building design, to ensure physical separation of the raw milk receival area and the production room.

Two methods for acidification of the whey are used in the manufacture of Ricotta. The preferred method, resulting in a creamier Ricotta, involves the utilisation of a yoghurt / whey mixture (mixed 1:4 v/v), which has a pH of approximately 4.4 – 4.6. The other method, used when there is no yoghurt available, is to add citric acid, mixed and dissolved 1:10 (v/v) in water. The former method appears to result in a product with a slightly higher pH, 6.4 as compared with 6.2 when acidified with citric acid. However, no differentiation was made between acidification methods for the purposes of stochastic modelling, with both methods used on a regular basis and data for both used to establish process parameters. Measurements taken for each production method did not demonstrate any significant difference between the two methods (data not shown).

5.3.1.2 Ricotta Food Safety Schemes

A food safety scheme was in the midst of implementation during this study, with the Tasmanian Dairy Industry Authority aiding in the design and application of the HACCP-based system. The system was designed so that both quality issues and food safety aspects were incorporated. Adaptations of the Process Flow Chart and Hazard Audit Table are shown in Tables 5.3 and 5.4 respectively. All points of the process are presented in Table 5.3, with the critical control points designated by the factory included in the Hazard Audit Table (Table 5.4). Included for consideration are any points which may introduce bacterial contamination, and therefore provide an avenue for *L. monocytogenes* to contaminate the cheese. The relevance of these CCPs, and possible other risk mitigation strategies for controlling *L. monocytogenes* will be objectively discussed, based on risk assessment outcomes.

5.3.2 Ricotta manufacture: Process Risk Model

A PRM was initially conducted utilising the assumptions listed in Chapter 2. Process parameters were assessed in terms of predicted *L. monocytogenes* growth by the Murphy-model selected for use in Chapter 3. In all cases, a normal distribution was used to describe the distribution for measured cheesemaking process parameters, as shown for each stage; production (Fig 5.1a-c), Final product attributes (Fig 5.8) and Storage and distribution (Fig 5.9) and Shelf life (Fig 5.11-5.15). Fitting statistics for temperature, pH and salt concentration inputs are presented in Appendix F.

Table 5.3 - Process Flow Chart for 1 kg Ricotta manufacture

⇒ Product transfer □ Inspection ○ Operation

Factory-designated Critical Control Points indicated in **bold**

□	**Whey Receival**
O	**Heat treatment**
O	**Milk addition**
O	Salt addition
O	Acidification
O	Settling time
O	Curd collection
O	Fill and top up moulds
⇒	Cool storage
□ O	**Pack and label**
⇒	Despatch

Adapted from D. Sandman (*pers comm.*, 1997)

Table 5.4 - Hazard Audit Table for 1kg Ricotta Manufacture

Step number / Operation	Potential Hazard	Critical Control Point	Preventative Control and Monitoring Procedure			Corrective Action
			Monitoring	Specification	Frequency recorded, responsibility	
1. Whey Receival	Sour/off flavour whey Poor Ricotta yield	Clean, fresh whey Volume of whey in vat	Sensory evaluation Titratable acidity Fill level	No off odours or flavours <0.12% Titratable acidity 100 mm from top of vat	Each vat Cheese make book Production manager	Reject the whey Do not overfill the vat
2. Milk addition	Microbial contamination Finished product quality	Prior to addition Measure milk volume each time	Sensory evaluation Titratable acidity Fill level	No objectionable odours <0.18% Titratable acidity 10% of whey volume	Each vat Cheese make book Production manager	Ensure fresh milk is used Adjust milk volume
3. Salt addition	Extraneous matter Off-flavours	Prior to addition Measure salt each time	Visual inspection Dose rate	Free from extraneous matter 600-700g/450L whey	Each vat Cheese make book Production manager	Sieve salt, adjust quantity accordingly Test cheese before release
4. Heat Treatment and agitation	Survival of pathogens Poor curd precipitation and coalescence. Low yield	Temperature Direct steam injection Thermometer accuracy	Measure temperature Visual observation Thermometer calibration	Heat to 83 to 88°C ± 1.0°C of reference thermometer	Each vat Production Manager 3 monthly	Determine source of problem, rectify and prevent recurrence. Adjust/replace thermometers
5. Acidification	Poor coagulation Incomplete precipitation Low yield	Addition of yoghurt or citric acid	Volume and pH of yoghurt / citric acid	Inoculum pH 4.4 to 4.6 pH of whey 5.3 to 5.5	Each vat Not recorded Factory staff	Check/adjust pH and volume of acidulant as appropriate
6. Scoop Curds	Contamination with spoilage and pathogenic microorganisms Low yield, poor curd quality	Effective hygiene and sanitation practices Curd recovery	GMP Visual observation	Clean sanitary equipment and practices. Collect discrete curd	Each vat Not recorded Factory staff	Avoid collecting cheese fines. Collect discrete curd particles. Observe GMP

Step number / Operation	Potential Hazard	Critical Control Point	Preventative Control and Monitoring Procedure			Corrective Action
			Monitoring	Specification	Frequency recorded	
7. Fill and top moulds	Microbial contamination	Effective hygiene and sanitation practices Mould filling	GMP Desired fill level	Clean sanitary equipment and practices. Slightly overfill moulds	Each vat Not recorded Factory staff	Reclean and sanitise all equipment. Fill moulds to desired level. Continually top up moulds.
8. Cool Storage	Product spoilage and growth of pathogenic microorganisms	Effective hygiene and sanitation practices Temperature	GMP Monitor and control coolroom temperature	Clean sanitary equipment and practices. Target 4°C maximum, range 2 to 6°C	Daily Coolroom temperature book Production manager	Reclean and sanitise all equipment and coolroom. Adjust temperatures accordingly
9. Packaging and labelling	Microbial contamination Incorrect weight Incorrect trade description	Hygiene & sanitation Level and zero scales Check weigh cheese Seal integrity Vacuum sealer Label information Coder	GMP Check scale accuracy before starting and at 15 minutes intervals Check weigh cheese at five minute intervals Vacuum level and heat bar settings Product description	Clean and sanitary ± 5g of reference weight Not less than the stated net weight and not more than 10% of the label weight. Complete seal Use-by date 28 days	Each unit Not recorded Factory staff	Identify and rectify problem Check and adjust scales to within scales to within 5 g of reference weight. Reject as far back as the latest documented scale check. Reweigh and relabel. Check accuracy of reference weight.
10. Despatch	Release of non-standard product Recall	Despatch details	Product & customer details	Coliforms <1/g *E. coli* <1/g CPS <100/g Environmental Listeria Product Listeria Use-by number of units customer details	Fortnightly Fortnightly Fortnightly Monthly Quarterly Each consignment	Isolate product. Determine source of problem and eliminate. Implement procedures in Listeria Manual or Product Recall Procedures

Adapted from D. Sandman (*pers comm*, 1997)

The fitted normal distributions and input parameter values for the stochastic modelling by the @RISK software are also presented in Appendix F.

5.3.2.1 Parameter interactions

Consideration was given to the measured cheesemaking parameters (Temperature, pH and water activity) and the likelihood of parameter interactions was determined to be minimal. The interaction of temperature and pH considered in Case Study 1 is not applicable for Ricotta because there are no starter cultures used in the manufacture of the product to influence pH. Therefore, alteration of pH and a_W is due to the addition of acidulant and other ingredients, and completely independent of temperature.

5.3.2.2 Ricotta production profile

Fresh whey cheeses are in excellent microbiological condition because of the high-temperature and long processing time involved in their manufacture (Papageorgiou *et al.,* 1996). The cooking process is a very consistent part of the Ricotta manufacture, as can be observed in Fig 5.1a. The variation observed between upper and lower limits was usually derived from the variability in the amount of whey storage time prior to use, or to a lesser degree, the rate of heating during the cooking process. At the point when the curd is scooped from the vat, it is essentially sterile, but due to the open nature of the cooling process, the cheese may be re-contaminated. The rate of curd cooling is controlled by the ambient temperature in the manufacture room, and the time at which the Ricotta is covered and transferred to the coolroom.

The production stage was modelled for the probability of potential *L. monocytogenes* growth for each four hour period following the cooking step. Growth profiling commenced at the point where the curd temperature had cooled to 40°C after being scooped from the vat.

Ricotta production (0-4 hr)

The freshly scooped Ricotta sits at room temperature for several hours, allowing the whey drainage to occur (Fig. 5.1a). The pH profile (Fig. 5.1b) shows the initial pH of the whey- milk mixture, followed by a sudden drop when addition of the acidulant occurs. Once the curd is formed, the lower pH whey is drained off and the pH returns to a value of ~ 6.3. Stabilisation of the pH takes place during the following hours as the remaining whey drains from the cheese and very little change occurs during the next 22 hours prior to packaging. The addition of salt during the cooking

results in a localised increase in salt concentration, reflected by the spike in Fig. 5.1c, but as the salt dissolves within the whey mass, the final salt concentration of the cheese is not significantly altered.

The highest risk segment of the manufacture process is the initial portion of the cooling curve, just after the Ricotta curd has been scooped from the vat. The curd remains at an ideal temperature for *L. monocytogenes* growth for an extended period of time. Fig 5.2a shows that the most likely outcome for the first four hours of the process is up to 1 log of predicted *L. monocytogenes* growth (95th percentile = 1.00 log). It can be seen from the shape of the predicted growth curve that the conditions are favourable for the proliferation of *L. monocytogenes*, as the curve is strongly weighted towards higher levels of growth. Temperature (c = 0.8611) and pH (c = -0.2456) were shown to be the most significant parameters affecting *L. monocytogenes* growth during the first four hour period (Fig 5.2b).

Ricotta production (4-8 hr)

After four hours of sitting at ambient temperature, the curd has cooled to 14.1 ± 4.7°C (n = 10), and by the 8th hr, the product temperature has dropped to 4.6 ± 3.1°C (n = 10). The mean level of predicted *L. monocytogenes* growth for this period is log 0.22 (50th percentile = log 0.18, 95th percentile = log 0.56) (Fig 5.3a). The sensitivity analysis (Fig 5.3b) shows the predominant controlling factor for growth is temperature (c = 0.99). It can be seen that pH is no longer inhibitory (c = 0.02), as most residual whey has drained off by this time. Salt concentration (c = 0.004) was found to be of little significance.

Ricotta production (8-12 hr)

The product is stored within the coolroom for the entire period, therefore the amount of predicted growth is less than in previous stages. Fig 5.4a shows that less than one generation of growth is possible for the entire four hour period, with the maximum predicted value 0.32 (50th percentile = log 0.041, 95th percentile = log 0.11). The sensitivity analysis (Fig 5.4b) shows that the model outcomes are totally correlated to temperature (c = 0.99), with the other parameters having small correlation values (pH = 0.08; %NaCl = 0.005).

Figure 5.2 – Modelled probability of potential *L. monocytogenes* growth during 0-4 hr stage of production and analysis of sensitivity to input variables

5.2a **5.2b**

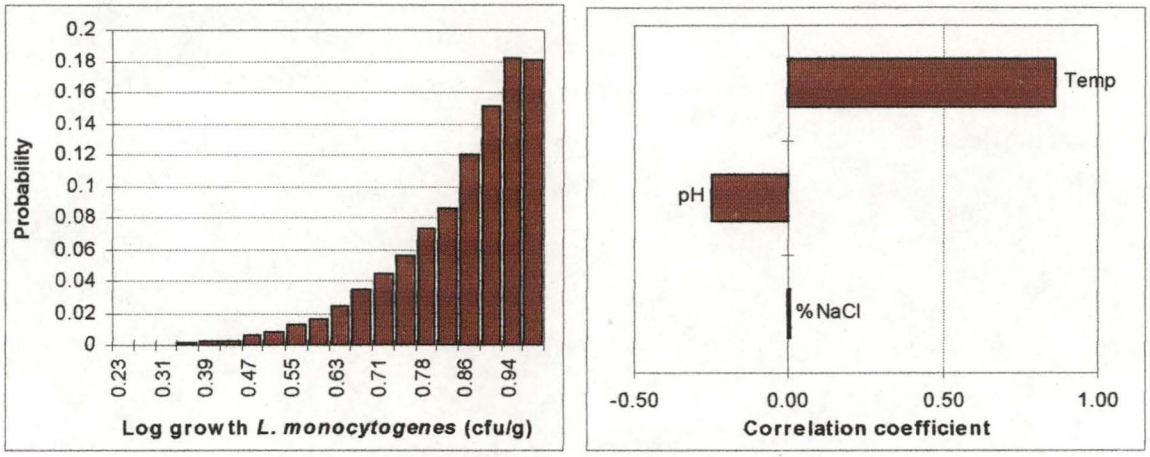


Figure 5.3 – Modelled probability of potential *L. monocytogenes* growth during 4-8 hr stage of production and analysis of sensitivity to input variables

5.3a **5.3b**

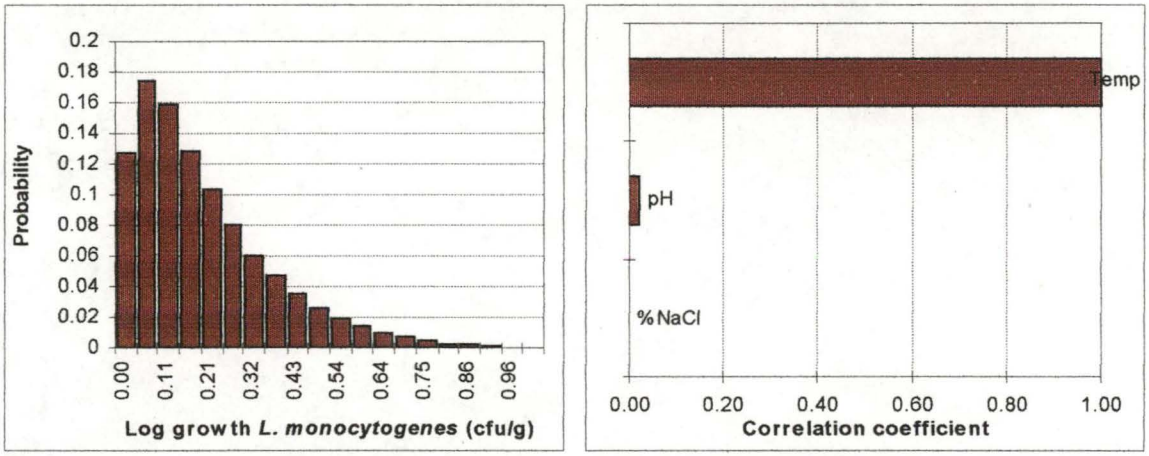


Figure 5.4 – Modelled probability of potential *L. monocytogenes* growth during 8-12 hr stage of production and analysis of sensitivity to input variables

5.4a **5.4b**

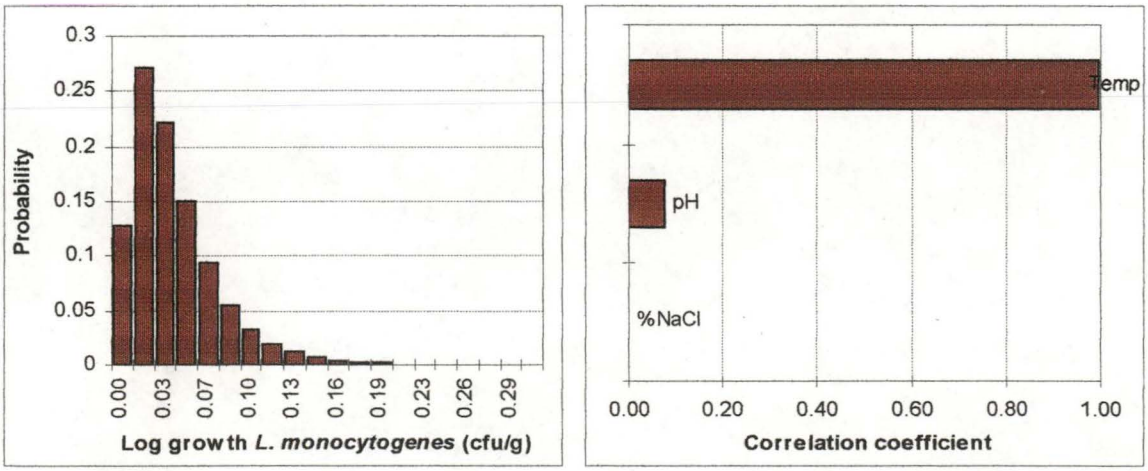


Figure 5.5 - Probability of potential *L. monocytogenes* growth and analysis of sensitivity to input variables during 12-16 hr stage of production

5.5a **5.5b**

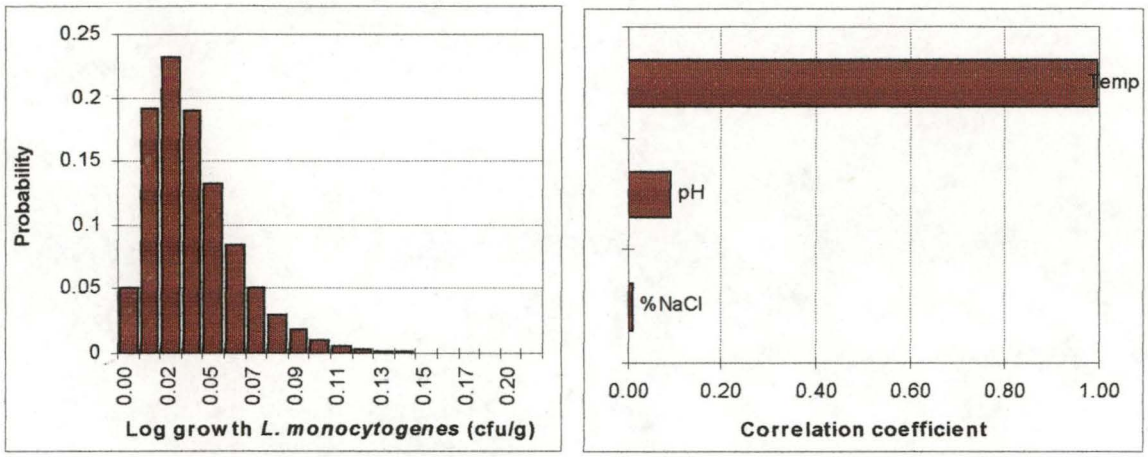


Figure 5.6 - Probability of potential *L. monocytogenes* growth and analysis of sensitivity to input variables during 16-20 hr stage of production

5.6a **5.6b**

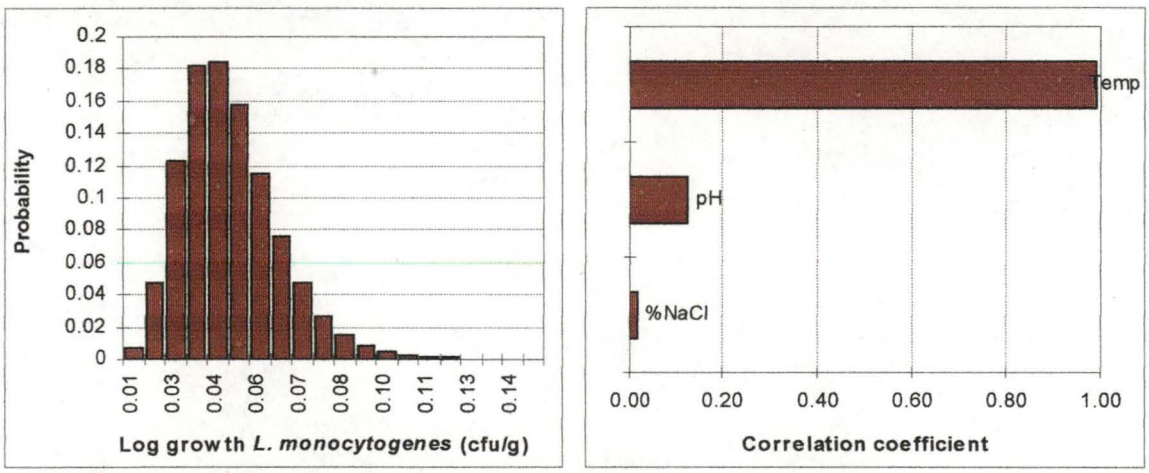
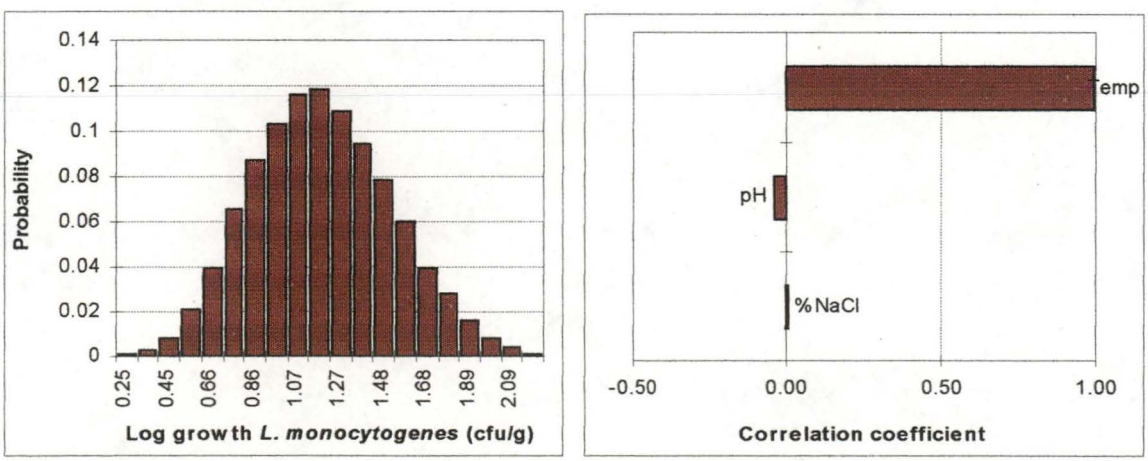


Figure 5.7 - Probability of potential *L. monocytogenes* growth and analysis of sensitivity to input variables during total production stage (0-24hr)

5.7a **5.7b**



Ricotta production (12-16 hr)

As for the previous four hours, the amount of predicted growth is restricted by the coolroom temperature (Fig 5.5a), with the mean level of growth predicted at log 0.041 (50^{th} percentile = log 0.036, 95^{th} percentile = log 0.084), with a mean generation time in the order of 26hr predicted. The sensitivity analysis (Fig 5.5b) again demonstrates temperature to be the dominant controlling factor (c = 0.99), with the pH value not substantially altering and the correlation remaining basically the same (c = 0.09). Salt concentration was not a factor influencing the model outcomes (c = 0.007).

Ricotta production (16-20 hr)

Early the next morning, the Ricotta is removed from the coolroom ready for vacuum packing into Cryovac barrier bags (Appendix A). The cheese may be exposed to ambient temperature for up to an hour while packaging is taking place, but no significant rise in product temperature was observed at the conclusion of vacuum packing (product temperature = 3.7 ± 0.9°C, n = 10).

This vacuum packaging process usually occurs early in the day, prior to the startup of equipment, such as the pasteuriser, which normally heat up the production room. Therefore, the ambient temperature would be unlikely to be of such magnitude as to extensively alter product temperature. This is reflected in the model predictions for *L. monocytogenes* growth, which are very similar to the previous four hours (Fig 5.6a). The maximum predicted outcome is less than half a generation of growth (maximum = log 0.15). Temperature (c = 0.99) is again shown to be the most highly correlated parameter with the model outcomes by the sensitivity analysis (Fig 5.6b)

The finished product is briefly immersed (2-3 secs) into boiling water to heat shrink the bags to the shape of the cheese. This time is so brief that it has little effect on the temperature of the 1 kg bulk cheese. When a similar process was conducted with much smaller cheese packages used for the Challenge tests outlined in Chapter 2, no adverse effect was observed on the level of *L. monocytogenes* inoculum.

Ricotta production - totals

The possible outcomes and correlations for the entire production process are shown in Figs 5.7a and 5.7b. The maximum *L. monocytogenes* growth predicted by the model was greater than 2 logs (50^{th} percentile = log 1.22, 95^{th} percentile = log 1.80). The sensitivity analysis shows that temperature is the dominant controlling factor (c = 0.99)

for *L. monocytogenes* growth, as was shown in each of the four hour summaries. Calculated salt concentration and pH are shown to have little influence on growth.

5.3.2.3 Ricotta final product attributes

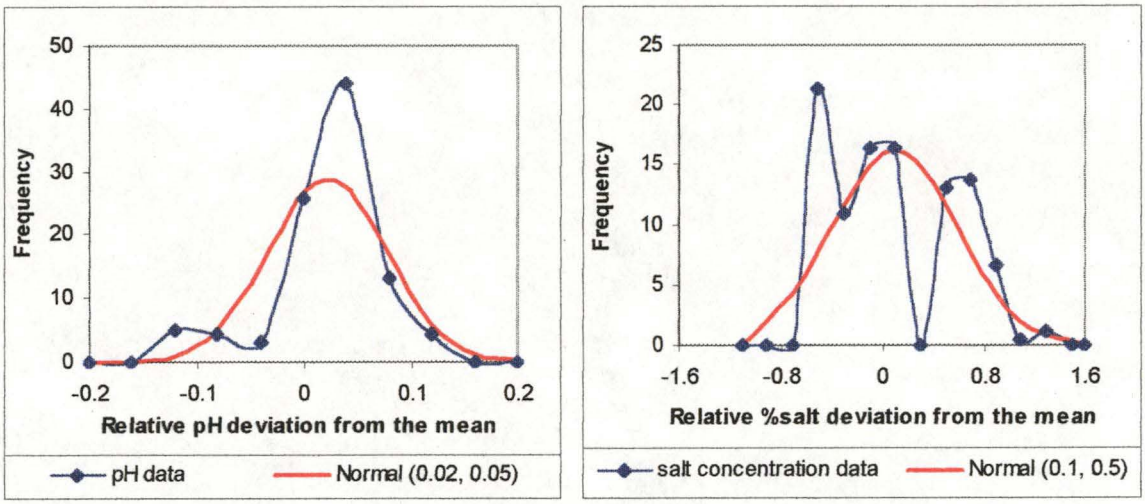
The final-product attributes are listed in Table 5.5. The pH value was found to be variable with an average value of 6.33 ± 0.32 (n = 126), ranging from 5.56 to 6.91. The distribution of pH values found in the end-product testing is shown in Fig 5.8. The two different methods of whey acidification may explain the wide range of observed pH values. A normal curve was found to adequately describe the distribution of pH values (Fitting statistics in Appendix F).

Water activity values were much more stable, the average a_w value was found to be 0.996 ± 0.002 (n = 175), with maximum of 0.999, and a minimum of 0.990. The distribution of salt concentration values (calculated from measured a_w values) is shown in Fig 5.8. The distinct peaks observed in Fig 5.8 may be an artifact of the conversion from a_w to salt concentration values. Because the a_w was very high, there were only a few values of a_w that could be measured – i.e. 0.990, 0.991, 0.992, 0.994 etc, as the resolution of the water activity meter is to three significant figures. The conversion to salt concentration leads to distinct values, and should the fitting software choose an intermediate value, then there is no way this can be modelled and the frequency is 0. This was not noted for Brie, although Fig 4.23 in the Brie Case Study does appear to have two peaks. The range of a_w values observed in the Brie was much wider than measured in the Ricotta, and a wider a_w range may tend to obscure this artifact.

Table 5.5 - Final-product attributes for 1 kg Ricotta

	pH	aw	SPC (log cfu/g)	Yeast (log cfu/g)	Mould (log cfu/g)
mean	6.33	0.996	5.15	4.93	4.56
SD	0.32	0.002	1.09	0.58	0.66
High	6.91	0.999	6.58	5.85	5.90
Low	5.56	0.990	3.10	4.41	4.00

Figure 5.8 - Comparison of Ricotta end-product pH values and calculated salt concentration and Comparison with Normal distribution selected by Bestfit software



The Ricotta was also examined for microbiological quality, by standard plate count, and yeasts and mould count. A wide range of contamination was observed, with a greater than 3 log difference between the minimum and maximum standard plate count (Table 5.5). Yeast and mould numbers were consistent, but rather high considering the severe heat treatment and the predominant source would therefore be airborne contamination. Ten samples of Ricotta cheese were also examined for the presence of pathogenic bacteria, according to the methods outlined in Chapter 2, with no positive isolations occurring.

5.3.2.4 Ricotta storage and distribution

A small proportion of product is sold direct to the consumer from the factory premises, being kept in a chilled display cabinet which runs at ~4°C. For the remainder, the cheese is stored in the factory coolroom, ready for distribution. No Ricotta is exported interstate owing to the delicate nature of the product and the 28 day shelf life. Transport times are therefore relatively short, with the product only distributed within the state of Tasmania. On occasions, transport of the product takes place in insulated vehicles, without refrigeration. In these cases, the onus lies on the manufacturer to ensure the product is at the minimum possible temperature when picked up by the distributor.

The conditions of storage and distribution are presented in Fig 5.9 (n = 10), which shows that the product temperature is well maintained throughout the cold chain. The product cools down to ~3°C after 24 hr, and thereafter the average product

temperature lies between 3-4°C, with the upper limit for product temperature being 6°C. Fig 5.9 also shows the fit of a normal distribution to the temperature data, with outlying values indicative of one distribution run where the storage temperature was unusually high.

Figure 5.9 - Mean Factory 'B' Storage and distribution temperatures (—) with upper and lower limits (—) for Ricotta and Comparison with Normal distribution selected by Bestfit software

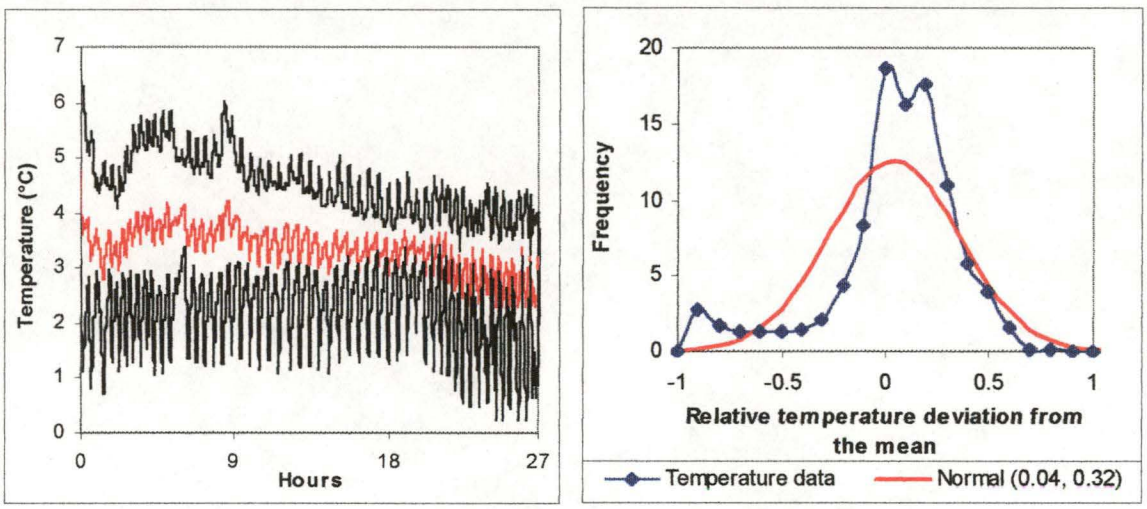
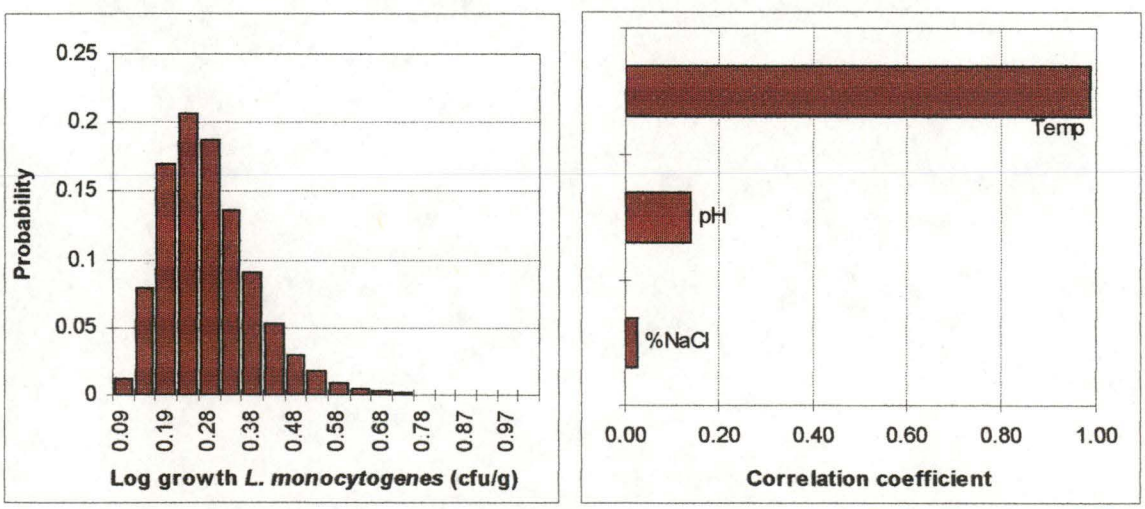


Figure 5.10 – Modelled probability of potential *L. monocytogenes* growth during Factory 'B' storage and distribution and analysis of sensitivities to input variables

5.10a **5.10b**



The predicted *L. monocytogenes* growth during the 27 hours of storage and distribution is shown in Fig 5.10a, with the mean predicted level of *L. monocytogenes* growth equating to approximately one generation (50^{th} percentile = log 0.29). The maximum predicted outcome is a log 1.07 increase, however the 95^{th} percentile = log 0.50. On examination of the model outputs, the outcome of greater than a one log increase was one iteration out of the ten thousand (ie P = 0.0001) included in the generation of model outputs. The sensitivity analysis (Fig 5.10b) showed the storage temperature to be the dominant controlling factor for *L. monocytogenes* growth (c = 0.99), with salt concentration and pH having little influence on growth.

5.3.2.5 Ricotta Shelf life

The shelf life of 28 days was determined by the manufacturer who assessed the cheese for taste and organoleptic properties at that time and deemed them to be acceptable, but on the verge of spoilage (A. Matteo *pers comm.,* 1997). The product remains acceptable within this timeframe due to the oxygen being removed by vacuum packing. However, once the vacuum seal is breached, the product rapidly spoils within several days, the signs of spoilage including odour, dryness and discolouration. Pintado & Malcata (2000) found the major spoilage organisms present in Ricotta throughout storage to be *Pseudomonas*, *Bacillus* and lactic acid bacteria. Thus, vacuum packaging is effective in the inhibition of some spoilage bacteria. However, it was demonstrated in Chapter 2 that the presence of an anaerobic atmosphere has little effect on the growth rate of *L. monocytogenes*. No differential was therefore made within the risk assessment model for the time when the product was subject to aerobic or anaerobic atmosphere.

The cheese was monitored during the shelf life of 28 days at storage temperatures of 5 and 10°C, to observe changes in pH and a_w which may affect the potential level of *L. monocytogenes* growth. Storage of the cheese at 5°C resulted in the pH dropping to ~5 at the conclusion of the shelf life, with a lower limit of 4.70 (Fig 5.11). The distribution for the pH development during the shelf life is shown in Fig 5.11, described by a normal distribution. At 10°C, the pH of the cheese reduced much more quickly, reaching a low value of ~ 4.5 (Fig 5.14). This is similar to the observations of Papageorgiou *et al.* (1996), who found Greek whey cheese varieties dropped from an initial pH value of between 6.30 - 6.50 at the end of production, to between 5.30 to 4.97 during storage. The a_w (and corresponding salt concentration)

did not significantly alter during the shelf life of the cheese at either 5°C (Fig 5.12) or 10°C (Fig 5.15).

The favourable conditions within the cheese are best exemplified by the predicted *L. monocytogenes* growth at the two storage temperatures examined. At 5°C, the model predicts a mean 9.11 log increase over the duration of the shelf life (50^{th} percentile = log 8.97,.95^{th} percentile = log 12.17). This increased to a 11.31 log increase with shelf life storage at 10°C (50^{th} percentile = log 11.25, 95^{th} percentile = log 13.66). This again demonstrated the necessity of specifying a maximum population density in the detailed risk assessment model. However, these results from the simple analysis demonstrate that although there is a slight increase in potential growth at the higher storage temperature, it is much less than expected. This is mainly due to the rapid acidity development at 10°C (Fig 5.14) due to the onset of spoilage, and serving as a barrier to *L. monocytogenes* growth.

Microbiological profiling of Ricotta during the shelf life at 5°C showed the standard plate count did not reach the maximum level until the halfway point of the shelf life, day 15 (Fig 5.17). This corresponded to the development of acidity due to the beginning of spoilage observed in Fig 5.11. At 10°C, total bacterial numbers increased quickly (Fig 5.18), up to a maximum level of ~ 10^9 cfu/g after 5 days. This caused the rapid onset of spoilage and the development of acidity observed in Fig 5.14. Therefore, although the risk assessment model was a simplification, in that it did not account for any possible inhibitions of *L. monocytogenes* from the natural cheese microbiota, the model did predict *L. monocytogenes* growth based on the changes in pH and a_w that the spoilage organisms were responsible for.

The next stage of the Case study was to predict an estimate of the likelihood of listeriosis as a result of consumption of Factory 'B' Ricotta cheese. Results from the model, including values from assumed parameters, are included in the following section.

Figure 5.11 - Mean pH during Ricotta shelf life at 5°C (—), upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software

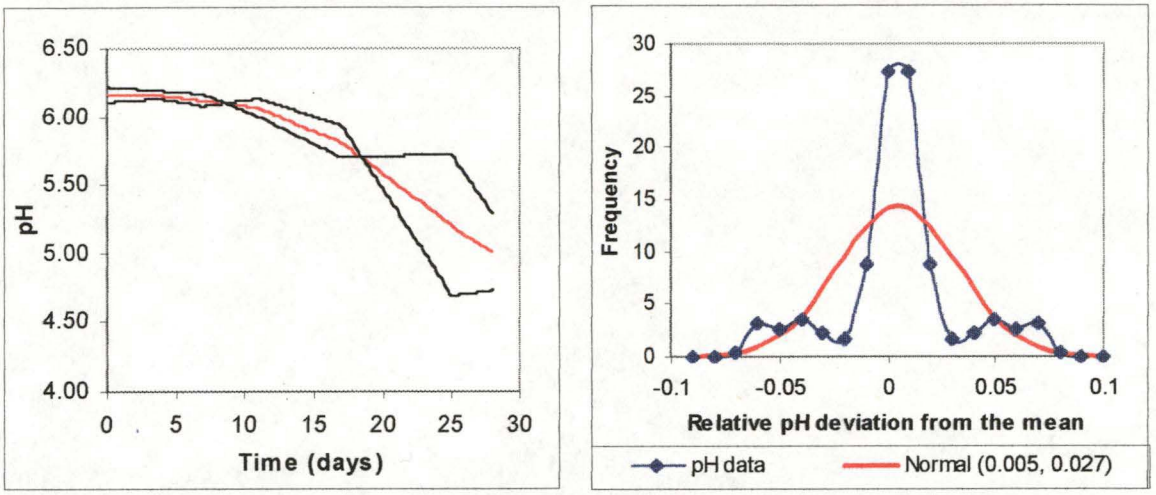


Figure 5.12 - Mean salt concentration during Ricotta shelf life at 5°C (—), upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software

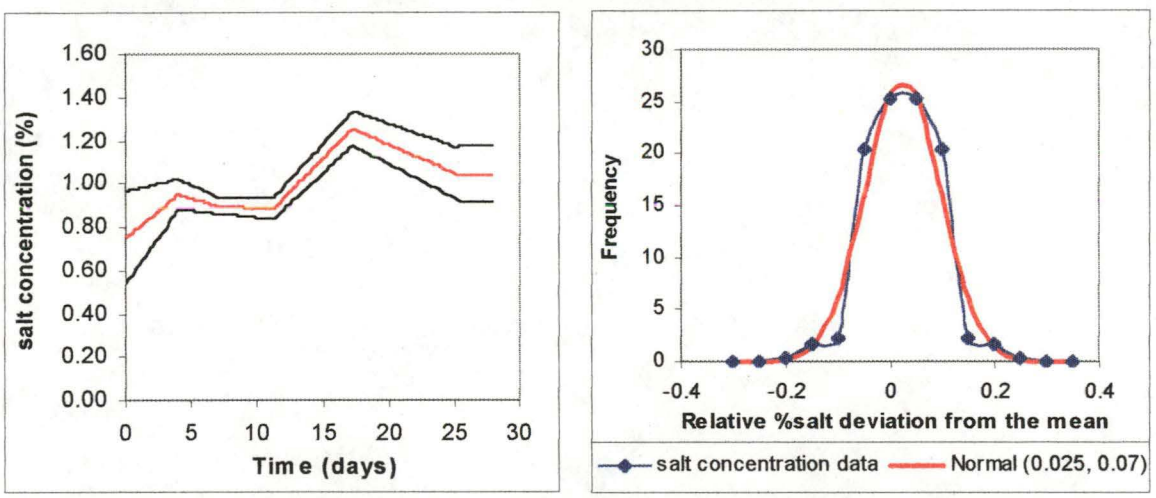


Figure 5.13 - Probability of potential *L. monocytogenes* growth and sensitivity analysis during Ricotta shelf life at 5°C

5.13a **5.13b**

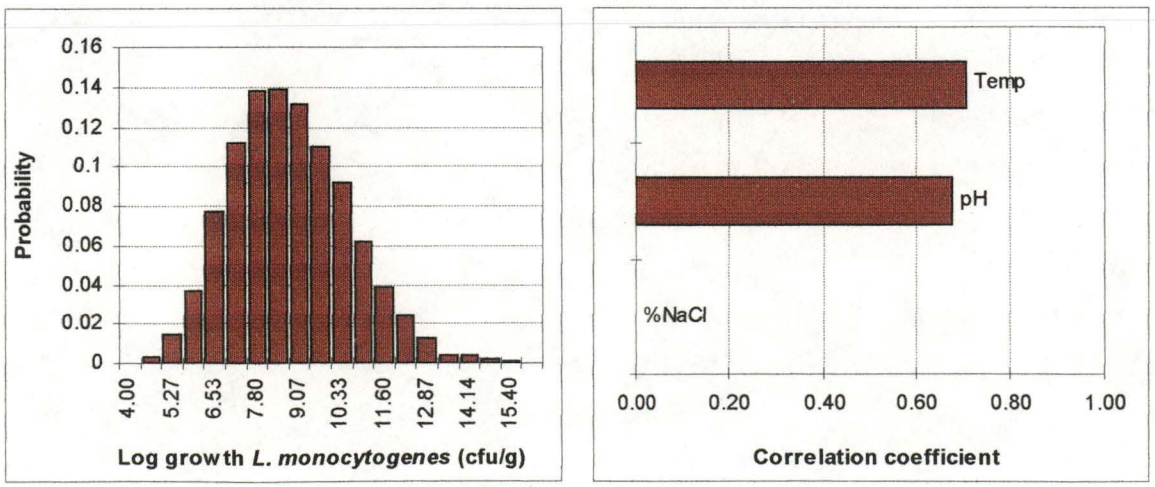


Figure 5.14 - Mean pH during Ricotta shelf life at 10°C (—), with upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software

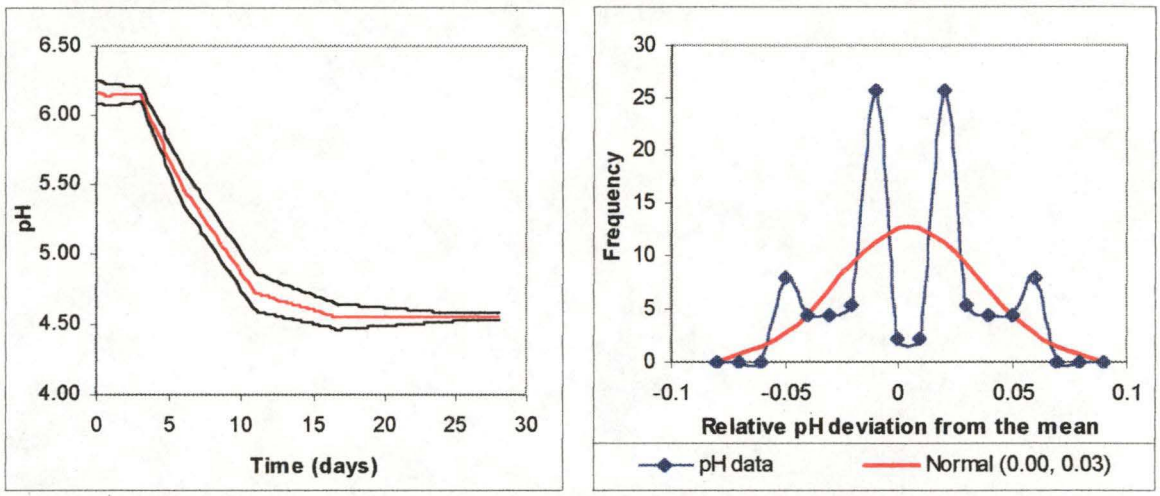


Figure 5.15 - Mean salt concentration during Ricotta shelf life at 10°C (—), with upper and lower limits (—) and Comparison with Normal distribution selected by Bestfit software

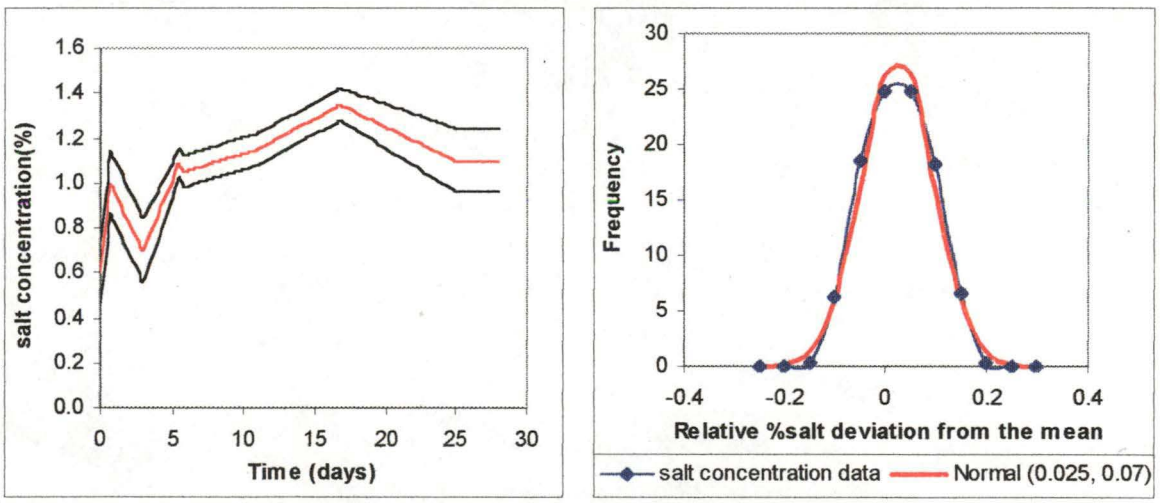


Figure 5.16 - Probability of potential *L. monocytogenes* growth and sensitivity analysis during Ricotta shelf life at 10°C

5.16a **5.16b**

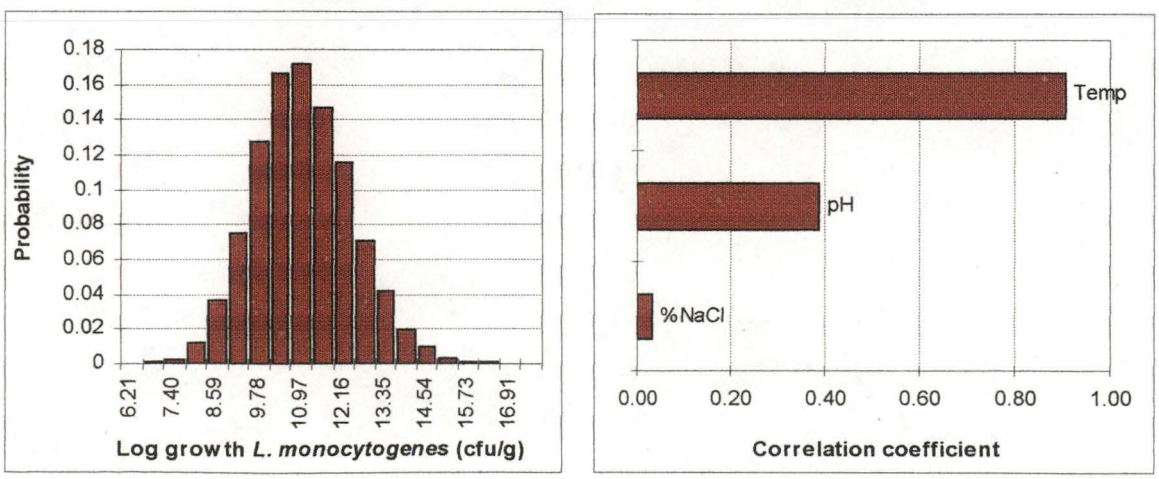


Figure 5.17 - Microbiological profile of 1kg Ricotta during shelf life storage at 5°C

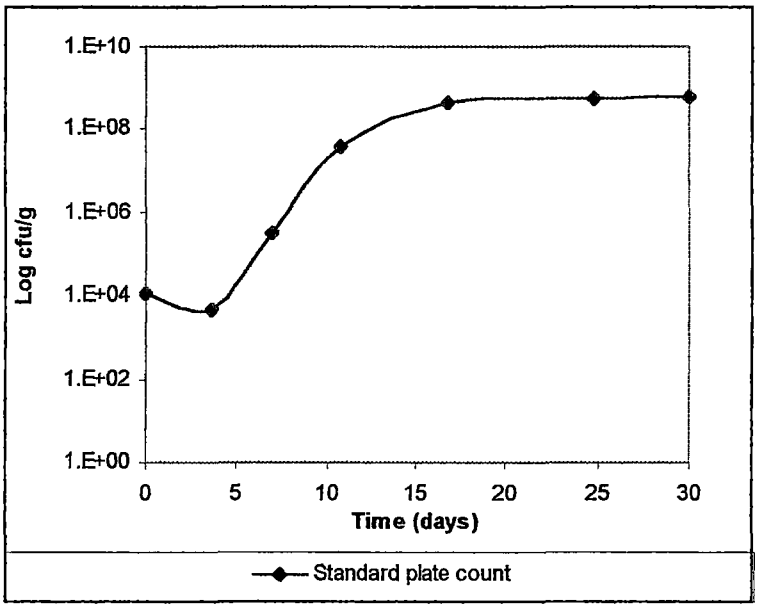
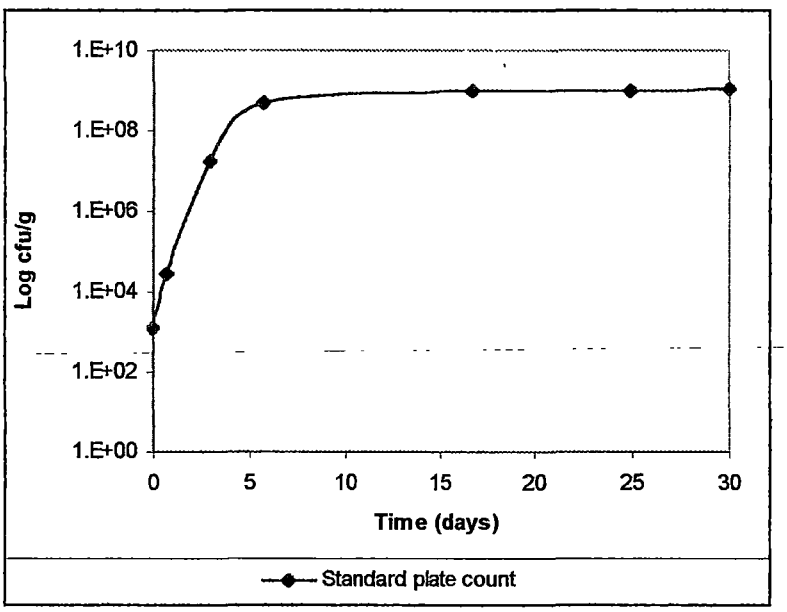


Figure 5.18 - Microbiological profile of 1kg Ricotta during shelf life storage at 10°C

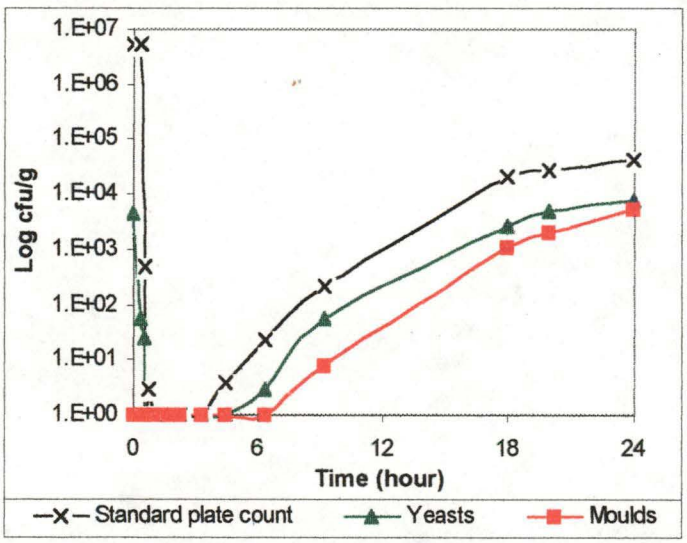


5.3.3 Ricotta detailed Quantitative risk assessment

5.3.3.1 Time of contamination

The microbiological profile of the Ricotta manufacturing process (Fig 5.19) showed the majority of contamination occurred during the cooling phase of the process. The exposed way in which the product cools results in it being very vulnerable to contamination with many microorganisms. The production room is usually wet, and if dust seeps in, then air contamination of the cheese surfaces accentuates the problem. The Ricotta is packed into perforated containers and left in a cool room to drain overnight, before being repacked into sealed containers. Condensation may form in the cool room and can fall onto the exposed product. Whey draining from one shelf can fall onto exposed product below and contaminate the product. Slow cooling, the high moisture content of the cheese, and the ability of the heated cheese curd to support the growth of microorganisms soon lead to a high count.

Figure 5.19 - Typical microbiological profile of Ricotta manufacture



Evaluation of the airborne contamination rate (Table 5.6) showed that rate of contamination in the production room was more than ten-fold the rate of contamination that occurred in the coolroom. The cheese may be located within the production room for up to 7 hours. This rate of contamination could lead to significant contamination of the cheese surface in hoops which are 20 cm in diameter. Therefore, although the rate of contamination may differ during different stages, it was considered that due to the open nature of the cheesemaking process, the probability of *L. monocytogenes* contamination was uniform throughout the process.

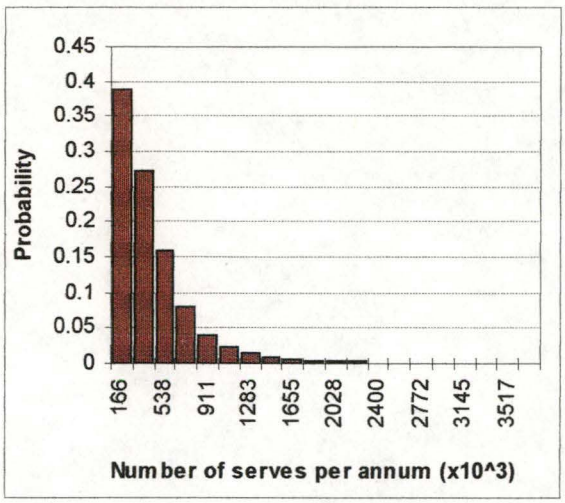
Table 5.6 - Measured airborne contamination rates during Ricotta manufacture

Ricotta cooling in production room	
Moulds (cfu/cm²/hr)	**Bacteria (cfu/cm²/hr)**
0.0233	0.1835
0.0167	0.2869
0.0167	0.2235
0.0050	0.2152
Ricotta storage in coolroom	
0.0050	0.0834
0.0083	0.0250
0.0050	0.0183
0.0067	0.0050
0.0100	0.0100

5.3.3.2 Number of serves

The calculated number of serves of Factory 'B' Ricotta *per annum* in Tasmania is shown in Fig 5.20, the mean calculated value was 522,057 (50th percentile = 416,410, 95th percentile = 1,194,817). This compares well with US consumption figures (USDA, 2001), where the number of meals of cottage, ricotta and cream cheese per annum is 5.10 x 10^9. Therefore, on a *per capita* basis, this calculates to consumption of Ricotta totaling approximately 3,400,000 meals per annum in a population the size of Tasmania. Since Factory 'B' Ricotta accounts for only 20% of market share, the number of serves calculates to approximately 680,000, similar to the figure estimated above. Fig 5.20 was thought to be unusual in that it does not appear to show a left hand tail, (i.e. the number of servings based on larger serving sizes). Examination of the percentile values (Appendix F) show that these values have been incorporate into the large 'spike' at the left hand of the figure.

Figure 5.20 – Number of Ricotta servings *per annum* in state of Tasmania



5.3.3.3 Level of L. monocytogenes at end of storage and distribution

The outcomes for the level of *L. monocytogenes* at the end of storage and distribution shows a very similar distribution to the Triangular distribution specified for the contamination level, and this is reflected in the sensitivity analysis (c = 0.988) (Fig 5.22). This indicates that little growth occurs during the production and storage stages (Fig 5.21). The predicted levels correspond almost directly with the initial contamination level, with the minimum value showing no growth (minimum = -2.97), and the mean and maximum values shifted upwards by almost one log, to reflect possible growth during the manufacturing and storage phases (mean = -0.27, maximum = 3.92). It was shown in the results of the semi-quantitative risk assessment that the majority of *L. monocytogenes* growth occurs during the initial four hours of the process. Should the contamination occur after this period (ie hours 4-17, and due to the uniform distribution specified for time of contamination, there is an 76% chance of it doing so), then very little growth will result. However, the sensitivity analysis did not show that contamination time (c = -0.052) and lag phase (c = -0.035) were strongly correlated with model outcomes. Although they were ranked second and third behind the level of contamination, the correlation values were very low. Production temperature was ranked fourth, but again had a low correlation value (c = 0.031).

Figure 5.21 – Predicted level of *L. monocytogenes* at end of storage and distribution stage for contaminated Ricotta cheese

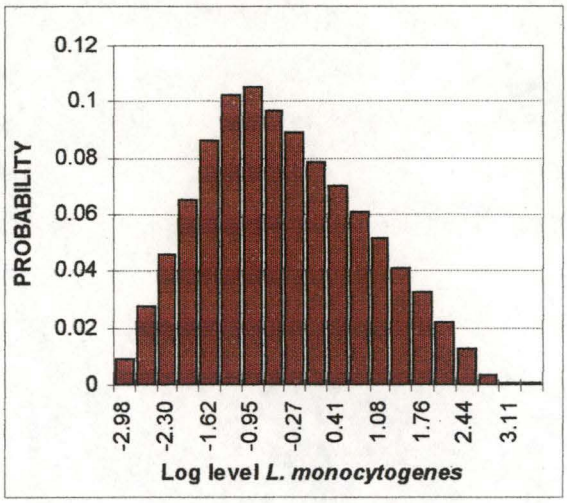
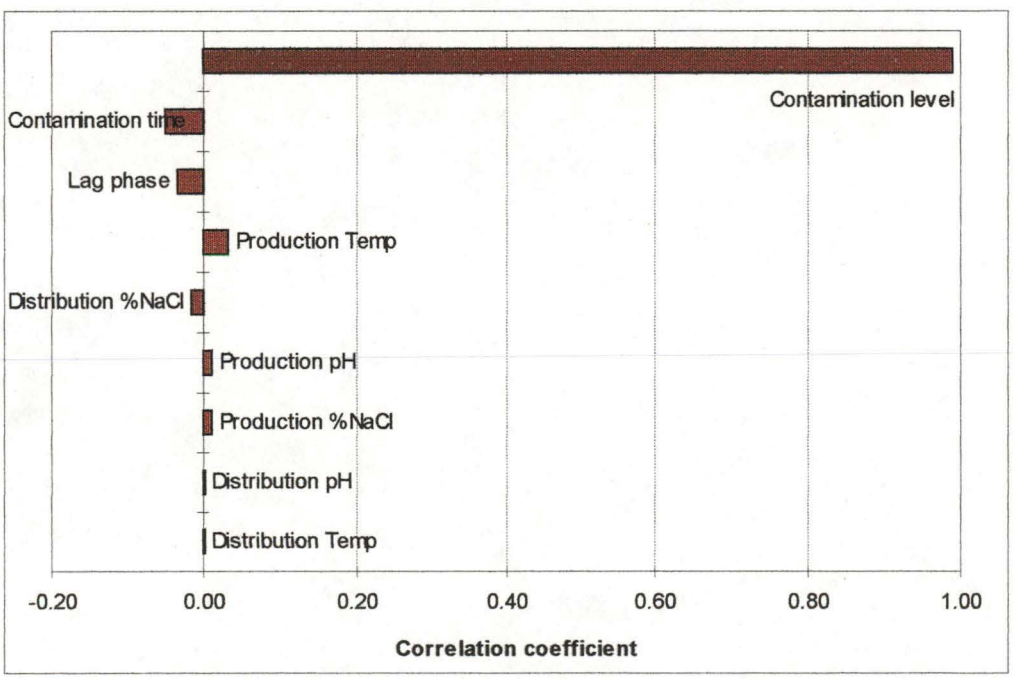


Figure 5.22 – Sensitivity analysis of model inputs for predicted level of *L. monocytogenes* in Ricotta cheese at end of storage and distribution



5.3.3.4 Level of L. monocytogenes at time of consumption

The large amount of potential *L. monocytogenes* growth which can occur during the cheese shelf life was shown in Section 5.3.2.5. Fig 5.21 showed that very little growth had occurred at the end of storage, however there is ample opportunity for the organism to grow to infectious levels prior to consumption. The predicted level of *L. monocytogenes* present on the cheese at the point of consumption is shown in Fig 5.23. It can be seen that the high levels observed in the previous Brie Case Study (Chapter 4) are not as applicable to this product. Provided all the correct elements are in place, high levels of growth can be achieved, but in the majority of cases, much lower numbers are observed. The small 'spike' to the right hand side of Fig 5.23 is due to the model upper limit being set at 10^8cfu/g. This leads to any predicted values which would have been greater than 10^8cfu/g being grouped together. Without this upper limit, it would be expected that a long tail of low probability values (high *L. monocytogenes* levels) would be observed.

The sensitivity analysis (Fig 5.24) provides a much clearer picture of the elements affecting *L. monocytogenes* growth than the analysis at the end of storage. Consumption time, lag phase duration, domestic handling and storage temperature and contamination time were calculated to be the most important elements. The initial contamination level, the most important factor to the end of storage, was found to be of little significance for this output, since even very low levels of contamination may lead to high numbers of *L. monocytogenes* in the final product due to the favourable conditions present in the cheese during the shelf life. Given the predicted levels at the end of storage and distribution, it appears the majority of this growth occurs during the product shelf life. The sensitivity analysis for the model outcomes (Fig 5.24) shows the significant parameters determining the level of *L. monocytogenes* in the cheese, with the time of consumption the most significant. As in the previous Case Study, the importance of assumptions in determining risk assessment outcomes is shown here again, with the two most significant parameters (consumption time c = 0.88 and lag phase c = -0.37) based on estimates.

Domestic handling of the cheese was shown to be an important determinant of risk. Domestic holding temperature was found to be the most third most important factor, and the cheese pH during domestic handling was shown to the 5th most significant factor. It was shown in the analysis of the cheese shelf life (Section 5.3.2.5) that the development of acidity in the cheese due to the onset of spoilage is a contributing factor in limiting *L. monocytogenes* growth.

Figure 5.23 – Predicted level of *L. monocytogenes* at end of time of consumption for contaminated Ricotta cheese

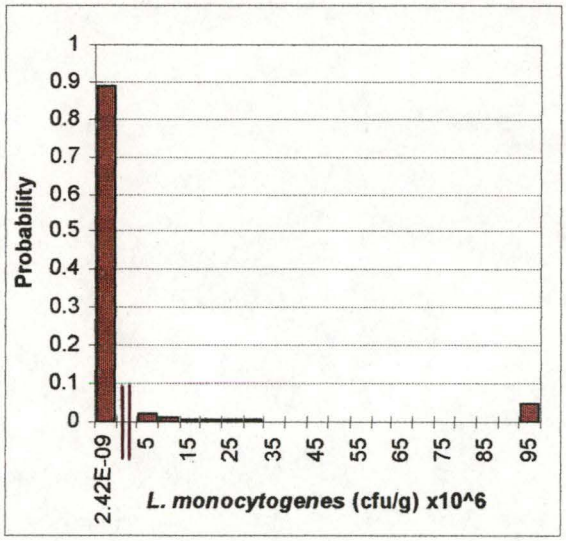
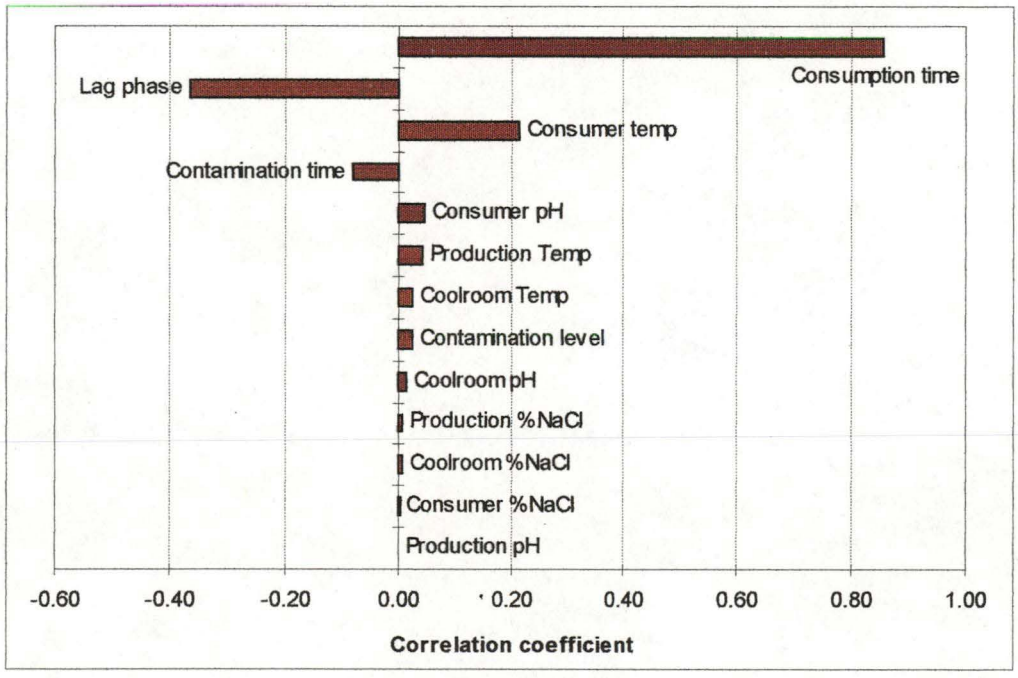


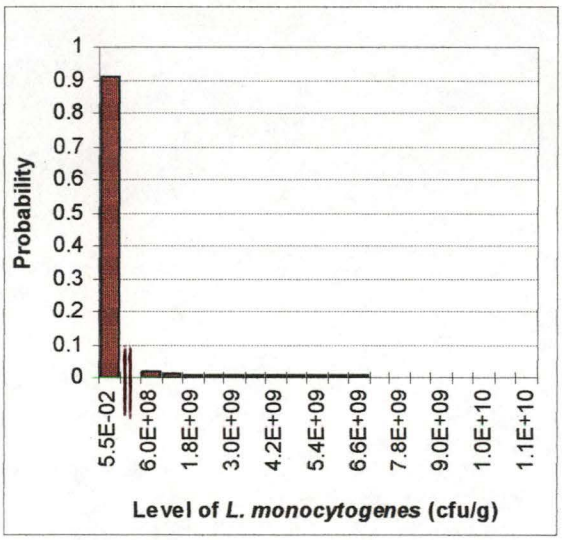
Figure 5.24 – Sensitivity analysis of model inputs for predicted level of *L. monocytogenes* in Ricotta cheese at time of consumption



5.3.3.5 Dose of L. monocytogenes

Following on from the level of *L. monocytogenes* on the cheese at the time of consumption, Fig 5.25 shows the predicted dose of *L. monocytogenes* presented to the consumer. As shown in Chapter 2, Dose was calculated by multiplying the level of *L. monocytogenes* (cfu/g) by the serving size of the cheese (g). The sensitivity analysis (not shown) for Dose is almost identical to Fig 5.24.

Figure 5.25 - Predicted dose of *L. monocytogenes* on a serving of cheese.



5.3.3.6 Probability of infection per meal

As in the previous Case Study, the two R-values were compared to generate a value for the probability of infection per meal. Once again, the R-value proposed by Buchanan *et al.* (1997a) gave estimates of listeriosis cases in the thousands (results not shown). Therefore the R-value proposed by Ross (*unpublished*) was utilised for all calculations. Using this R-value, the probability of infection per meal estimated from the consumption of Factory 'B' 1 kg Ricotta was calculated according to Eqn 2.2. The probability of infection from consumption of contaminated Ricotta ranged from 2.44×10^{-15} to 2.22×10^{-4} (mean value = 5.28×10^{-6}).

5.3.3.7 Predicted number of listeriosis cases per annum

Predictions of listeriosis cases were generated for the susceptible and general population groups of Tasmania. In general, the number of cases was predicted to be very low. The mean number of predicted cases for the general population would result in one listeriosis case in 67 years; this rate was one in 10 years for the susceptible population. However, it was also shown from the maximum predicted values that the cheese may be the cause of a total up to 4.2 cases of listeriosis in just one year (95^{th} percentile = 0.8). This outcome was assumed to be due to the highest contamination frequency

5.3.3.8 Alteration of input parameter estimates

Effect of storage temperature

A simulation was generated in which the domestic storage temperature input value was set at 10 ± 0.5°C for the duration of the cheese shelf life. Section 5.3.2.4 showed that the alteration of this storage temperature did not have as big an impact as expected, due to the development of corresponding acidity. The mean predicted level of *L. monocytogenes* at time of consumption increased to 6.32×10^{7} cfu/g (45^{th} percentile = 1×10^{8} cfu/g), which was reflected in the relative estimates of listeriosis risk. The mean number of total listeriosis cases was estimated at 1.01 (50^{th} percentile = 0.828) for the susceptible population and 0.15 (50^{th} percentile = 0.119) for the general population, a ten-fold increase in the number of cases estimated from consumer storage at 5°C. The mean probability of infection per meal also increased 12-fold, to 6.32×10^{-5}. These simulation results underline the importance of storage temperature in limiting the potential growth of *L. monocytogenes*.

Effect of limiting shelf life

A simulation was generated in which the shelf life of the product was limited by one week, to 21 days. The level of *L. monocytogenes* at the time of consumption was limited to a mean level of 1.42×10^{5} cfu/g (50^{th} percentile = 373 cfu/g, 95^{th} percentile = 3.1×10^{5} cfu/g). This had the effect of reducing the mean probability of infection to 1.45×10^{-7}, and the total number of listeriosis case in Tasmania to 0.01 per year (50^{th} percentile = 4.04×10^{-6}, 95^{th} percentile = 1.57×10^{-3}) (Full results presented in Appendix F). This element of risk would present one case of listeriosis in 100 years. It would be assumed that this level of risk would be acceptable to the manufacturer.

Figure 5.26 – Predicted distribution of Listeriosis cases per annum in Tasmania as a result of Ricotta consumption a) general population; b) susceptible population Contamination frequency Triangular (0, 0.003, 0.043)

5.26a **5.26b**

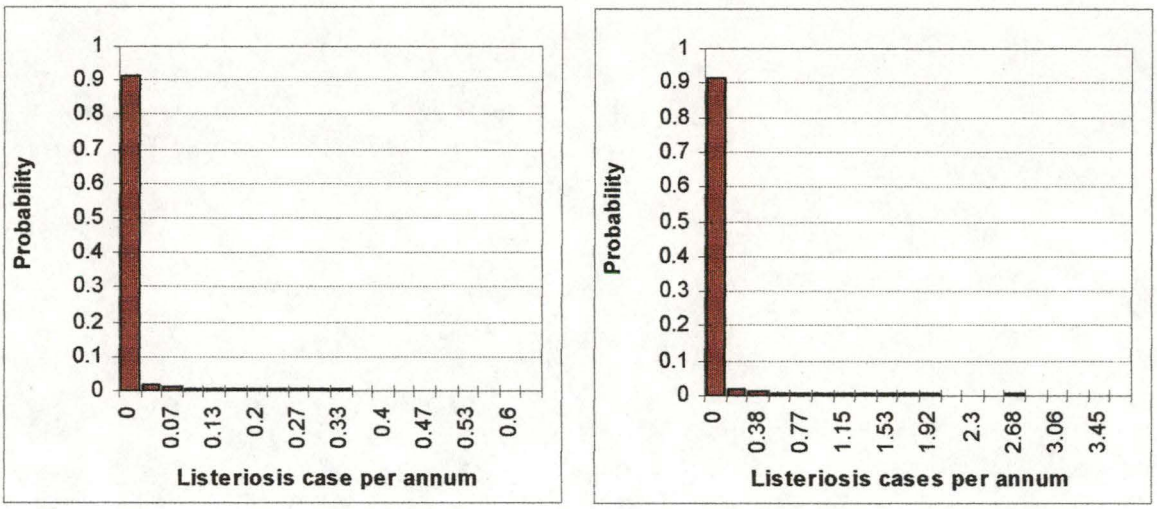


Figure 5.27 – Sensitivity analysis for model inputs for risk of listeriosis from Factory 'B' Ricotta *per annum*

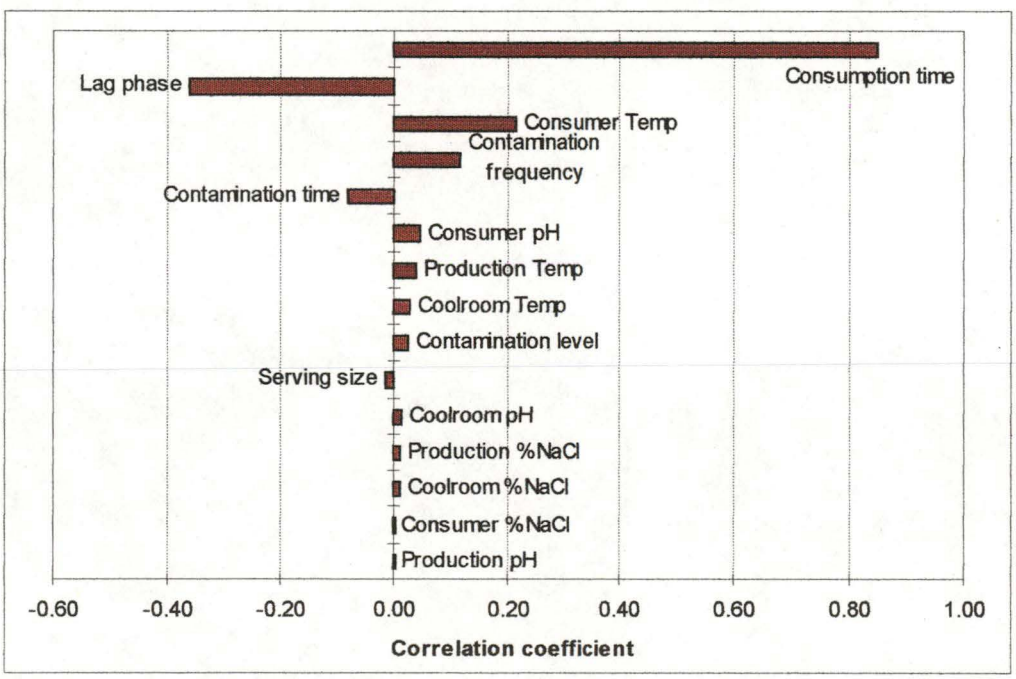


Table 5.7 – Predicted number of listeriosis cases per annum predicted from consumption of Factory 'B' Ricotta in Tasmania, based on age and susceptibility

Tasmania					
	Minimum	**Mean**	**Maximum**	**50th percentile**	**95th percentile**
Cancer	0	4.33e-2	0.156	1.62e-6	2.94e-2
Transplant	0	1.37e-2	0.494	5.11e-6	9.29e-2
AIDS	0	1.08e-2	0.391	4.04e-6	0.073
Diabetes	0	2.25e-2	0.813	8.41e-6	0.153
Pregnant	0	2.90e-2	1.047	1.08e-5	0.197
Kidney	0	8.38e-4	3.03e-2	3.13e-7	5.69e-3
> 60 years	0	1.86e-2	0.672	6.96e-6	0.126
< 30 days	0	3.16e-3	0.114	1.18e-6	0.021
Susceptible population Total	**0**	**0.103**	**3.717**	**3.85e-5**	**0.699**
1 – 9 years	0	5.96e-4	2.15e-2	2.23e-7	4.05e-3
10 – 19 years	0	6.93e-4	2.50e-2	2.59e-7	4.71e-3
20 – 29 years	0	2.24e-3	8.09e-2	8.37e-7	1.52e-2
30 – 39 years	0	6.30e-3	0.227	2.35e-6	4.28e-2
40 – 49 years	0	3.35e-3	0.121	1.25e-6	2.28e-2
50 – 59 years	0	4.78e-3	0.173	1.79e-6	0.032
General population total	**0**	**0.015**	**0.53**	**5.52e-6**	**0.100**

5.4 Discussion

Due to the harsh nature of the cooking process, it would be expected that the microbial load on Ricotta would be very low. To ensure the microbiological safety of the Ricotta, elimination of *L. monocytogenes* is reliant upon the cooking process. There is very little risk of *L. monocytogenes* surviving the cooking process, as the time and temperatures used are well in excess of the minimum batch pasteurisation requirements (ANZFA, 1999). This is a true CCP, as the time/temperature combination used for cooking the product would be able to eliminate any hazard formed beforehand through the addition of ingredients contaminated with *L. monocytogenes*. There is also a large safety margin included in the cook, thus it would be unlikely for the critical limits to ever be breached. The initial motivation for designating the whey receival as a CCP was for quality-related issues, with the potential hazard listed in Table 5.4 as "sour/off flavour and poor yield". The effective kill step within the cooking process renders the designation of whey receival as a CCP (Table 5.3) inappropriate, as any *L. monocytogenes* growth which did occur during the receival and handling of whey would be inactivated.

The addition of milk to the whey was also designated as a CCP. Due to the reasons listed above, this is again inappropriate, as the cooking step would inactivate any *L. monocytogenes* which may be present in the milk. As an aside which is beyond the scope of this thesis, this step may be an appropriate CCP for the control of coagulase positive staphylococci, as the toxin formed by these organisms is very heat resistant and may not be inactivated by the cooking step. The method of adding the milk should be included in GMP as a control point, to avoid spillage of the raw milk onto the sides of the vat. This is one of several possible sources within the Ricotta manufacturing process which may contribute to post-cooking contamination. These include recontamination of the whey from inadequately heat-treated raw ingredients, airborne contamination during the cooling phase, and contamination from handling during packaging. Packing and labelling has also been designated as a CCP by the factory (Table 5.3), and while this is vitally important in being able to trace the product should a fault be discovered, it is not able to eliminate the hazard from the product.

The Factory 'B' brand Ricotta was produced in an open vat, therefore the potential hazard exists for the recontamination of whey by raw milk or any other contaminated ingredient which may splash onto the side of the vat and not be subjected to the high cooking temperatures. It was this logic, as well as quality-related

issues which initially led the factory to designate milk addition as a CCP (Table 5.4), as contaminated matter may then drip back onto the freshly scooped Ricotta curd. However, this step is more suitable to be included in GMP procedures, as this not a critical step in eliminating the hazard. The risk of potential contamination can also be lowered by following recommendations made for batch pasteurisation by the Australian Dairy Authorities Standards Committee (ADASC, 1999a). Use of a covered vat with a heater to heat the air in the head space above the whey to minimum pasteurisation temperatures can greatly reduce the risk of contamination.

Airborne contamination was shown to be a source of adulteration while the product was cooling (Table 5.6) in the production room. Several remedies to this problem are available. Adequate filtration of air entering the production room, the use of air locks, and positive air pressure within the production room to ensure that air moves from the production room to the outside, avoiding the intake of unfiltered air (ADASC, 1999b). Since the findings of this study, production practices have been changed to limit contamination by covering the Ricotta with a sanitised plastic covering after scooping of the curds has been completed. This slows the cooling rate of the Ricotta by not allowing the heat to escape as quickly, but also limits the potential for airborne contamination. This procedural change took place at the conclusion of this study, and it was unknown at the time of writing what effect (if any) this change had on product quality.

The final possible source of contamination arises due to the manual method in which the product is packaged. The use of hygienic practices is necessary during handling and packing of this product. Papageorgiou *et al.* (1996) recommended post-packing pasteurisation of the cheese to minimise the risk posed by *L. monocytogenes*. Provided GMP principals are employed, the level of contamination at this stage should be minimal. No obvious rise in microbial load was detected between packaging and the final product. Venables (1989) also highlighted risk factors associated with Ricotta manufacture. The re-usable containers used for packaging Ricotta may not always be cleaned properly between uses, therefore it must be ensured the hoops are sanitised between each batch to avoid cross contamination. The Ricotta is packed into perforated containers and left in the coolroom to drain overnight, before being packed into sealed containers. Condensation may develop in the coolroom and fall onto the exposed product. Whey draining from one shelf to another can fall onto exposed product below and contaminate that product. Excessive

handling of the product is a significant factor, and in many cases the finished product is not stored satisfactorily.

Examination of the model outcomes from the PRM analysis present a definitive strategy for limiting the potential growth of *L. monocytogenes*. A mean predicted 1.22 log increase in the production stage (Fig 5.7a) (50^{th} percentile = log 1.22, 95^{th} percentile = log 1.80) is mostly due to the initial four hour period. On average, approximately two-thirds of the predicted growth occurs in the first four hours. Results also showed that *L. monocytogenes* growth is almost solely correlated to temperature during Ricotta manufacture (c = 0.99), with pH (c = -0.04) and salt (c = 0.009) not greatly influencing model outcomes. Therefore, the ideal solution would be to ensure the cheese cools as rapidly as possible, to limit the timeframe in which the curd remains at favourable temperatures for *L. monocytogenes* growth. There are, however, limitations on how quickly the product can be cooled. To ensure maximum expulsion of whey, resulting in a product that does not contain excess moisture and an acidic taste, the curd must remain soft and loosely knitted (A. Matteo *pers comm.*, 1997). If the curd was to be placed in the coolroom at an early stage, the curd may compress too quickly and entrap surplus whey.

Section 5.3.1.1 outlined the two forms of acidification used in the production of Ricotta, citric acid and a yoghurt / whey mixture, and an apparent difference in pH of the final product. During the production stage, once the curds are scooped, a large amount of residual whey remains with the curd, which proceeds to drain over the following several hours. The PRM showed that the whey acidity has a slight inhibitory effect on predicted *L. monocytogenes* growth, which is signified by the negative correlation value for pH for the first 4 hours of the production stage. This inhibitory effect becomes less as the whey drains from the curd. Thus, although there is a slight difference in pH between the two methods of acidification, with citric acid resulting in a slightly more acidic product, this provides a slight additional barrier to *L. monocytogenes* growth. There may also be an additional inhibitory effect on *L. monocytogenes* growth dependent on which acidulant is added. The addition of yoghurt would result in lactic acid being the predominant acidulant, which has a different inhibitory effect to the direct addition of citric acid (El-Shenawy & Marth, 1989). The effect of the undissociated form of organic acids on *L. monocytogenes* is further discussed in Chapter 6. The PRM demonstrated that the initial four hours of the production stage provide the most favourable conditions for *L. monocytogenes* growth, therefore any factor which slows growth during this time, will have a larger

relative reduction in *L. monocytogenes* risk, than steps taken at any other time during manufacture.

The modelling outcomes presented for Ricotta have graphically illustrated that the cheese presents no limiting factors to prevent the proliferation of *L. monocytogenes*. Any risk mitigation factor is primarily reliant on the storage temperature to limit the proliferation of *L. monocytogenes* to a manageable level. Datta *et al.* (1988) showed *L. monocytogenes* could reach levels up to 3.6×10^6 cfu/g on temperature-abused Ricotta. Additional test samples obtained from implicated factories (Venables, 1989) have contained levels of *L. monocytogenes* up to 1.5×10^6 cfu/g. Genigeorgis *et al.* (1991b) judged Ricotta the best cheese for *L. monocytogenes* growth, after challenge tests found growth occurred on all samples at incubation temperatures ranging from 4°C to 30°C. Observed growth varied from 1.5 logs to greater than 4 logs of growth. Papageorgiou *et al.* (1996) investigated the growth of *L. monocytogenes* on traditional Greek whey cheeses (similar in composition and manufacture methods to Ricotta), and found growth to occur readily at 5, 12 and 22°C. Levels up to 10^8 cfu/g were observed and generation times at 5°C were in the order of 16 hr. Results of this type led Davies *et al.* (1997) to suggest the use of nisin to prevent the growth of *L. monocytogenes* on Ricotta.

It can be seen from the results presented in this Case Study, that the consumption of Factory 'B' Ricotta presents less of a risk than in the previous Product Case Study for Brie. Much of this reduced risk level is due to the reduced level of consumption (4 tonnes), when compared to Brie (~120 tonnes). However, it can also be seen that should *L. monocytogenes* contaminate the product, and storage temperature is allowed to go unchecked, the organism is capable of growth to high levels. The risk assessment conducted in this Case Study accounts only for Ricotta that is consumed as a ready-to-eat (RTE) product, and is therefore likely to result in an over-estimation of risk, as some Ricotta is likely to be used in cooking. Dependent on the severity of cooking by the consumer, *L. monocytogenes* may be totally inactivated therefore providing no degree of risk. Considering it is difficult to assess what the end-use of the product will be (i.e. what proportion of Ricotta will be consumed as RTE, and what proportion will be used in cooking), it must be assumed that this risk assessment presents a worst-case scenario.

The sensitivity analysis shows that contamination frequency was only the fourth most important parameter for determining model outcomes. This was a surprising outcome. It demonstrates, however, that unlike Product Case Study 1, should contamination occur it is not a *fait accompli* that *L. monocytogenes* will grow to an infectious dose level and listeriosis result. The probability of *L. monocytogenes* reaching very high levels is much less in this Product Case Study than for Brie for a number of reasons. There is a much shorter period in which there is opportunity for contamination to occur. It was also shown in the PRM analysis results that the conditions exist for substantial growth to occur only in the initial four hours of the Ricotta manufacturing process. The storage and shelf life of Ricotta cheese is also approximately half that of the Brie. This gives *L. monocytogenes* much less time to multiply to high dose levels, and therefore the risk is less.

Several strategies for lowering the listeriosis risk for this product were presented. The importance of consumer handling was illustrated through the sensitivity analysis of the detailed risk assessment, where consumer temperature was shown to be the third most important parameter. The shelf life results did demonstrate however, that storage at a higher temperature has the effect of increasing the onset of spoilage, with a resultant development of acidity which had the effect of slowing relative *L. monocytogenes* growth. Despite the preservative effect of the acidity development, storage at 10°C was shown to still allow *L. monocytogenes* to increase to a level which increased the risk of listeriosis twelve-fold. Limiting the shelf life to 21 days was shown to decrease the mean probability of infection risk by 36 times.

5.4.1 Conclusions

Ricotta cheese should have a low microbial population, however due to the high pH and moisture of the product, any contaminating microorganisms has the potential to readily grow. Gross numbers on the finished product are indicative of contamination post-heat treatment, and the presence of poor sanitary packaging and storage conditions at the factory. This can be accomplished through application of sanitary filling practices, use of clean cooling water, filtering air in production and packaging rooms, and quick cooling of the cheese, as well as covering to limit the extent of airborne contaminants.

The only reliable method of assuring a safe Ricotta product is to ensure that effective heat treatment is employed, the only true CCP of the process based on the QMRA results, and that the product does not become re-contaminated after the heat

treatment. The risk assessment has demonstrated, that provided the frequency of contamination is low, then the risk posed by Factory 'B' Ricotta is low. Given the end use of Ricotta often involves it being cooked prior to consumption, this would lower the risk even more substantially.

6. PRODUCT CASE STUDY 3 – 1 KG MASCARPONE

6.1 Introduction

This Case Study presents a quantitative assessment on risk of listeriosis associated with the consumption of 1 kg Mascarpone cheese, also manufactured by Factory 'B'. It uses the same methods as described in the Case Studies presented in Chapters 4 and 5.

6.1.1 Mascarpone

Mascarpone is a fresh, unripened cheese resembling thick clotted cream. The cheese has a rich sweet, slightly acidic taste with a moist and creamy appearance. The cheese is packaged into plastic tubs, which are then further packed into Cryovac bags and vacuum-packed to extend the shelf life to 28 days.

Mascarpone originated in Lodi, near Milan, Italy, more than two centuries ago (Rogers, 1995). The cheese typically contains a maximum of 75% moisture and a minimum of 45% fat and no preservatives are permitted in the final product (ANZFA, 1999). The cheese is made from cream, which is coagulated due to a combination of acidification and high heat. The final cheese yield varies from 40 to 55%, dependent on the fat content of the cream used.

The Australian fresh cheese market has steadily increased over the last decade, owing to greater consumption of cream cheeses (Willman, 1998). Mascarpone is primarily consumed as a ready-to-eat product, used in appetisers, cheesecakes, desserts, or may be served with fruit. A popular use in Australia is within the dessert Tiramisu. It was the consumption of this dessert which led to a recall of Italian Mascarpone cheese due to the presence of *Clostridium botulinum* in the product during September 1996. The cheese was linked to the death of a 15 year old boy in Italy, and 7 other cases of botulism were recorded in the Naples region (Anon, 1996). All of these cases were linked to a single batch of Mascarpone and approximately 100,000 tubs of the cheese were removed from stores in Italy, Belgium, Spain, Sweden and Austria. Italian authorities alerted the Food and Drug Administration in the United States that some of the product may have been exported to the U.S. (USDA, press release P96-14). This incident served to highlight the fact that contamination with pathogenic bacteria can occur in this type of product, and result in severe illness.

6.2 Materials and Methods

6.2.1 Characterisation of the Mascarpone cheesemaking process

The Mascarpone manufacturing process was modelled using the methods described in Chapter 2. Process parameters consisting of temperature, pH and a_w (converted to equivalent salt concentration) were measured within the factory environment, commencing from receival of the cream through to packaging of the final product. For modelling purposes, process parameter distributions were defined as described in Chapters 4 and 5, commencing subsequent to the heat treatment, at the point where the curd temperature reached 40°C. Both 250g and 1kg sized Mascarpone are manufactured at Factory 'B'. However, the modelling process is applicable for all sized product, since it is manufactured as one batch and separated into the two package sized only when the final cheese is packed.

The manufacture process was arbitrarily divided into 4 hr (and one 5 hr) periods, to more accurately assess predicted *L. monocytogenes* growth over the 17 hrs of the production (Manufacturing) stage. Subsequent to the Manufacturing stage, the Storage and Transport stage (day 2) and Shelf Life stage (days 3-30) were assessed in terms of predicted *L. monocytogenes* growth. The transport data for Mascarpone was identical to that used for Product Case Study 2, as both the Ricotta and Mascarpone are manufactured on the same premises and distributed through the same system and operators.

6.2.2 Analysis of Mascarpone final product attributes

The shelf life specified by the manufacturer is 28 days from the day of production. The microbiological profile (standards plate count, lactic acid bacteria, yeasts and moulds) and intrinsic parameters (pH and a_w) of the final product were monitored at the conclusion of manufacturing by the methods described in Section 2.2.3, and also during the shelf life by the methods outlined previously.

6.2.3 Risk assessment – assumptions

The assumptions necessary for this risk assessment of Mascarpone cheese are listed in Table 6.1. These were used in conjunction with the assumptions already outlined in Chapter 2. The following section describes the assumptions used in the detailed risk assessment model to generate predicted health-based outcomes.

6.2.3.1 Frequency of contamination

There has been no detection of *L. monocytogenes* in this product since it had been manufactured at the factory (~ 2 years), although testing is only conducted on a 3-monthly basis. As stated in the previous Case Study, there had also been no positive environmental detections of *Listeria* within the factory. The ten samples used for microbiological analysis in this study were all negative for *L. monocytogenes*. Therefore, given the lack of available data to establish an incidence, the same distribution was used as specified in Product Case Study 1 (Triangular 0, 0.003, 0.043).

Table 6.1 - Distribution inputs for detailed Mascarpone risk assessment

Variable	Description	Unit	Distribution / Model
Time of contamination	Time during manufacture when cheese is contaminated	hr	Uniform (0, 17)
Time of consumption	Time during shelf life when cheese is consumed	hr	Triangular (45, 144, 720)

6.2.3.2 Time of contamination

A microbiological profile of the manufacturing process was conducted to determine the possible contamination sources and to determine whether the uniform distribution specified in Table 6.1 was appropriate. The results are shown in Section 6.3.3.1.

6.2.3.3 Time of consumption

The Mascarpone has a 'Use-by' date of 28 days, specified by the processor, from the manufacture date. As in the previous Case Study, a Triangular distribution was used to describe the time of consumption (Table 6.1). Since the finished product is a fresh, unripened cheese, it is very susceptible to spoilage, therefore, only packages which were kept intact would be still be acceptable at the end of the shelf life point. Rogers, (1995) stated that once the vacuum seal is breached, the cheese will last no longer than 5 days before spoilage occurs. The quantity of cheese manufactured is based on the number of orders (limiting the amount of wastage), and the 1 kg sized cheeses are mainly used in retail establishments. Therefore, it was assumed that a large proportion of the cheese will be consumed within the first week of the shelf life. This would severely limit the probability of consumption after the 'Use-by' date, however,

as in Product Case Study 2, an extra 2 days were allocated in the modelled shelf life for the cheese to be consumed.

6.2.3.4 Exposure assessment

This product has a very low rate of production at Factory 'B' with annual production estimated at 1.2 tonnes per year. The cheese is not exported interstate, and therefore all cheese is consumed within the state of Tasmania. The consumption rate of Mascarpone is not high, Section 2.5.2.2 shows that Mascarpone accounts for approximately 0.9% of total cheese consumption in Australia. Based on this percentage, the total consumption of Mascarpone in the state of Tasmania is estimated to be ~12 tonnes, resulting in Factory 'B' having a market share of 10%. This factor was used to limit the final estimate of listeriosis cases to only consumption of Factory 'B' Mascarpone.

6.3 Results

6.3.1 Characterisation of the Mascarpone manufacture process

Mascarpone is produced from full cream which is heated to a high temperature via direct steam injection, utilising a similar manufacture method to that detailed in the previous Case Study for Ricotta (Chapter 5). A typical manufacturing schedule is shown in Table 6.1. Tartaric acid is added to the cream, as it has a stronger and sharper taste than citric acid. The combination of heat (80-85°C for ~15 min) and lowered pH causes the cream to thicken and form a cream cheese. The holding time for the heating process is not as long as for Ricotta, but still well in excess of minimum heat treatment specifications for batch pasteurisation. The heat treatment is capable of inactivating most of the microbiota present within the cream, rendering the scooped curd virtually sterile when it is scooped from the vat and transferred to a cloth-lined mould. Contamination then may occur from the air while the product is cooling at room temperature, and when placed in the coolroom.

The cheese is packaged after 20 hr by scooping into plastic tubs holding either 1kg or 250g. The tub is then placed into a Cryovac barrier bag, and the entire container vacuum packaged. HACCP plans developed for this product will be applicable to both product sizes, as the cheese is produced in a single batch, and it is not until packaging that any difference in the process occurs. A typical manufacturing schedule for the production of Mascarpone cheese is shown in Table 6.2. Temperature, pH and calculated salt concentration profiles are shown in Figs 6.1a, 6.1b and 6.1c respectively. A normal distribution was used for modelling of process parameters

(Fitting statistics in Appendix G). Ten process data sets were modelled. The addition of more process data might have improved the fit of distributions.

Table 6.2 - Typical manufacturing schedule and parameter values for 1kg Mascarpone

Step	Time		Temp (°C)	pH	a_W
Cream receival	Day 1	11:00 AM	4.9 ± 0.8°C	6.57 ± 0.16	0.996 ± 0.001
Begin steam heating		11:35 AM	7.1 ± 0.6°C	6.37 ± 0.24	0.996 ± 0.001
Add salt		11:40 AM	15.1 ± 3.7°C	6.20 ± 0.21	0.995 ± 0.001
Add acidulant		11:45 AM	32.1 ± 5.7°C	6.01 ± 0.23	0.995 ± 0.001
Stop heat / agitation		12:00 PM	83.1 ± 2.3°C	5.95 ± 0.22	0.995 ± 0.001
Scoop curd		12:15 PM	76.2 ± 3.2°C	5.94 ± 0.21	0.995 ± 0.001
Cover with cloth		12:30 PM	70.5 ± 3.4°C	5.93 ± 0.20	0.994 ± 0.002
Move into coolroom		3:00 PM	24.2 ± 6.4°C	5.90 ± 0.22	0.994 ± 0.002
Vacuum-packed	Day 2	7:00 AM	3.2 ± 0.6°C	5.84 ± 0.25	0.993 ± 0.001

6.3.1.1 Mascarpone Ingredients

The list of ingredients for Mascarpone manufacture consists of raw cream, salt, and tartaric acid. These ingredients are added prior to the heat treatment, therefore presenting very little risk of contamination with *L. monocytogenes*. The only concern, already being highlighted in the Ricotta Case Study, arises from the lack of physical separation between the milk and cream receival and production areas. As stated in Chapter 5, this was being addressed at the time of writing.

6.3.1.2 Mascarpone Food Safety Schemes

As for Product Case Study 2, a food safety scheme was being implemented during this project, with the TDIA aiding in the application of a HACCP-based system. Adaptations of the Process Flow Chart and Hazard Audit Table are shown in Tables 6.3 and 6.4 respectively. Both quality issues and food safety aspects were incorporated into the system. The Hazard Audit Table (Table 6.4) has been used to highlight potential entry points for contamination into the final product.

Figure 6.1a - Mean temperatures for Mascarpone production (—),upper and lower limits (—) and Comparison with normal distribution selected by BestFit software

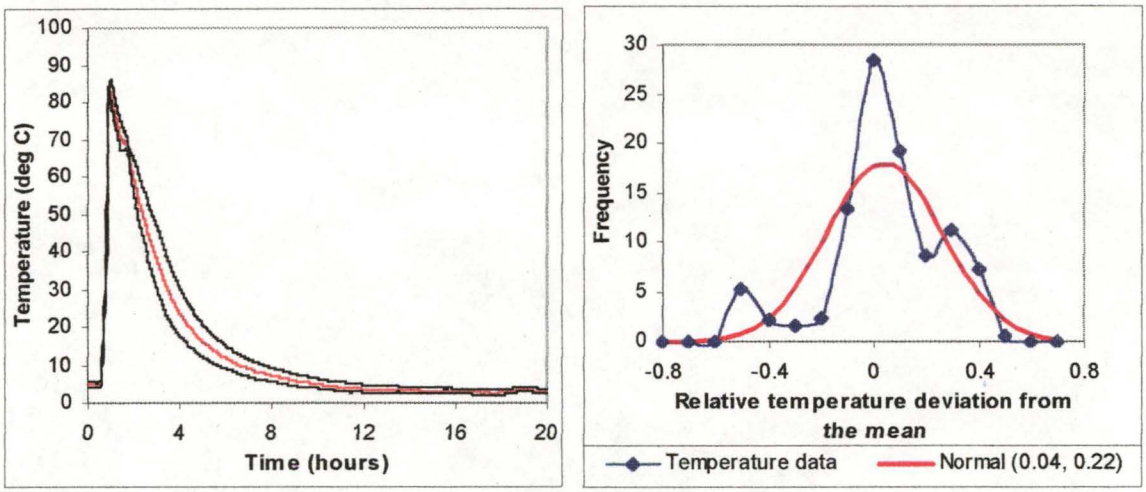


Figure 6.1b - Mean pH values for Mascarpone production (—),upper and lower limits (—) and Comparison with normal distribution selected by BestFit software

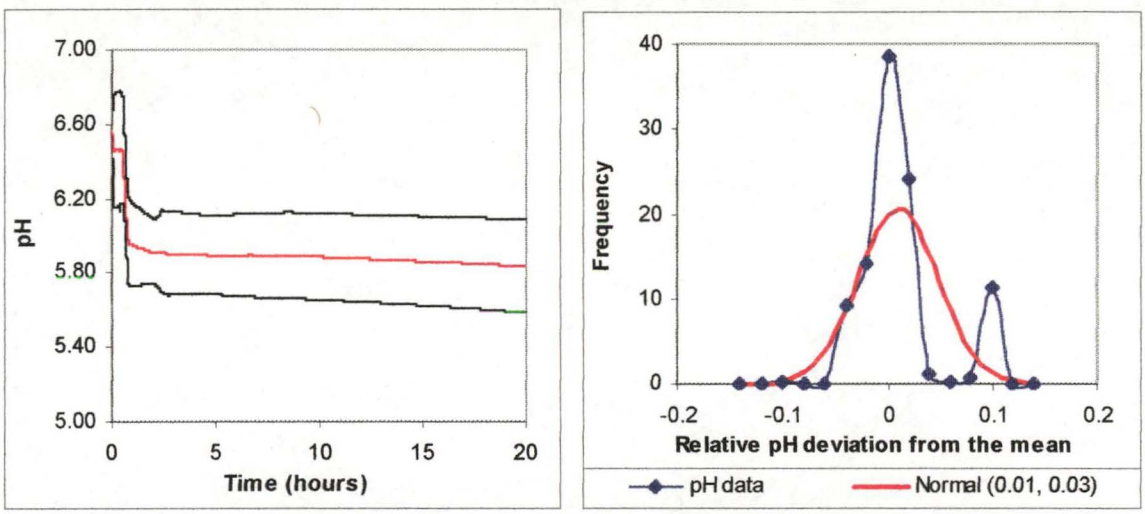


Figure 6.1c - Mean calculated salt concentration values for Mascarpone production (—),upper and lower limits (—) and Comparison with normal distribution selected by BestFit software

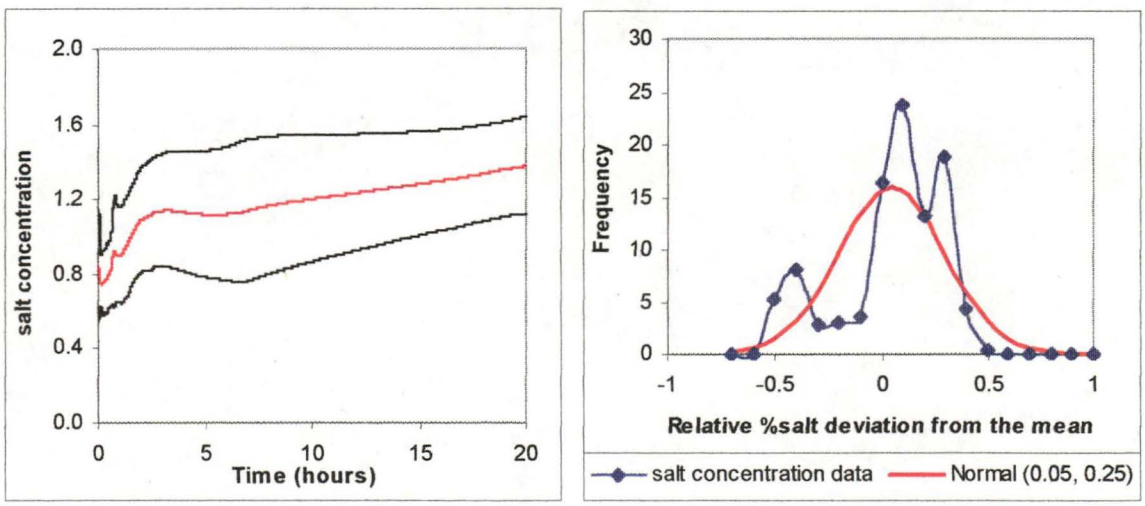


Table 6.3 - Process Flow Chart for 1 kg Mascarpone manufacture

⇒ Product transfer □ Inspection ○ Operation

Factory-designated Critical Control Points indicated in **bold**

□	**Cream Receival**
O	**Cream Storage**
O	Standardisation
O	**Heat treatment**
O	Salt addition
O	Acidification
O	Agitation
O	Settling time
O	Scoop curds
⇒	Cool storage
□ O	**Pack and label**
⇒	Despatch

Adapted from D. Sandman (*pers comm.*, 1997)

Table 6.4 - Hazard Audit Table for 1kg Mascarpone Manufacture

Step number / Operation	Potential Hazard	Critical Control Point	Preventative Control and Monitoring Procedure			Corrective Action
			Monitoring	Specification	Frequency recorded, responsibility	
1. Cream Receival	Contamination (Chemical, microbiological, and physical)	Clearance tests	Hygiene Volume Temperature Senses Test Titratable acidity Fat SPC	Clean and sanitary Actual <8°C Acceptable 0.17% maximum 45-50% <50,000 cfu/mL	Before accepting load Each load Milk receival book Factory operatives Fortnightly	Clean and sanitise equipment before receiving milk. Operator advises factory manager if cream is sub-standard. Factory manager decides to accept/reject
2. Storage	Contamination and growth of spoilage bacteria. Excessive agitation.	Sanitation Time Temperature Agitation method	Hygiene Storage time Temperature Agitation	Clean and sanitary >36 hours <8°C Continuous no froth	Before filling vat Each load Milk receival book Factory operatives	Clean vats, lines etc. Follow up on results. Process within 36 hours. Avoid frothing.
3. Heat Treatment	Survival of spoilage and pathogenic organisms	Effective cleaning Temperature Time Calibration of equipment	Visual inspection Temperature Time Indicating thermometery	Clean and sanitary 80 - 82°C 15 seconds minimum 80°C ± 1.0°C of reference thermometer	Before and after use Continuous – chart Not recorded Continuous – chart 6 monthly Temperatures noted and recorded every 30 minutes	Clan and sanitise before use Adjust controls to achieve operating conditions Chart and indicating thermometer to read ± 1.0°C of each other Adjust/replace thermometer
4. Acidification	Poor coagulation Low yield	Addition of tartaric acid	Volume, dilution of tartaric acid	20 g tartaric acid in 2L of water	Each vat Not recorded	Check/adjust of tartaric acid and method of addition

Step number / Operation	Potential Hazard	Critical Control Point	Preventative Control and Monitoring Procedure			Corrective Action
			Monitoring	Specification	Frequency recorded	
5. Scoop Curds	Contamination	Hygiene and sanitation	Visual inspection	GMP Clean, sanitised draining cloths and moulds	Each vat Not recorded Factory staff	Scoop discrete curd particles Follow GMP
6. Cool Storage	Contamination	Hygiene and sanitation Temperature	Visual inspection	GMP Target 2 to 4°C	Each vat Not recorded Factory staff	Follow GMP and. Adjust temperature accordingly
7. Packaging and labelling	Contamination Incorrect weight Incorrect trade description	Hygiene & sanitation Level and zero scales Check weigh cheese Seal integrity Vacuum sealer Label information Coder	GMP Check scale accuracy before starting and at 15 minutes intervals Check weigh cheese at five minute intervals Vacuum level and heat bar settings Product description	Clean and sanitary ± 5g of reference weight Not less than the stated net weight and not more than 10% of the label weight. Complete seal Use-by date 28 days	Each unit Not recorded Factory staff	Identify and rectify problem Check and adjust scales to within scales to within 5 g of reference weight. Reject as far back as the latest documented scale check. Reweigh and relabel. Check reference weight accuracy annually.
8. Despatch	Release of non-standard product Recall	Despatch details	Product & customer details	Coliforms <1/g E. coli <1/g *S. aureus* <100/g Environmental *Listeria* Product *Listeria* Use-by number of units customer details	Fortnightly Fortnightly Fortnightly Monthly Quarterly Each consignment	Isolate product. Determine source of problem and eliminate. Implement procedures in Listeria Manual or Product Recall Procedures

Adapted from D. Sandman (*pers comm.*, 1997)

6.3.2 Mascarpone manufacture: Process Risk Model

Using the assumptions listed in Chapter 2, a PRM was initially generated. Process parameters were converted into a level of *L. monocytogenes* growth through the use of the predictive model for *L. monocytogenes* growth by Murphy *et al.* (1996) selected for use in Chapter 3. A normal distribution was used to describe the distribution for measured cheesemaking process parameters, as shown for each stage; production (Fig 6.1a-c), Final product attributes (Fig 6.7a-b) and Shelf life (Figs 6.8 and 6.9). Fitting statistics for temperature, pH and calculated salt concentration inputs are presented in Appendix G.

6.3.2.1 Parameter interactions

Consideration was given to potential interaction between the measured cheesemaking parameters (temperature, pH and water activity) but no likelihood of parameter interactions was thought to be probable. As with Ricotta, there are no starter cultures present in the product to affect pH, with pH and a_W altered primarily through the addition of ingredients.

6.3.2.2 Mascarpone production profile

The severe heat treatment involved in Mascarpone manufacture, renders the freshly scooped curd in very good microbiological condition. Due to the low volumes of cream used in the production (~ 100 L), the heating process rapidly heats the cream to the maximum temperature (80-85°C). This is reflected in Fig 6.1a, where the upper and lower limits of the cooking stage are in very close proximity to the Mean value. The freshly scooped curd is placed into a cloth-lined hoop, where it is left to cool and the residual whey is allowed to drain. Once the Mascarpone curd has been scooped from the vat, the cloth is layered over the top of the curd to completely cover it. This has the effect of trapping in some heat. However, from Fig 6.1a this appears to have a negligible effect on the rate of cooling.

Once the acidulant is added to coagulate the curd, the pH does not vary significantly for the rest of the Production stage (Fig 6.1b). The addition of salt during the cooking stage has a small impact on calculated salt concentration, observed as a small 'hump' in Fig 6.1c at time ~ 1 hr of the process. Subsequent to this, the a_W was observed to slowly decrease during for the duration of the production stage (observed as a slow increase in calculated salt concentration), due to expulsion of the whey, and the resultant loss in curd moisture.

The probability of potential *L. monocytogenes* growth was modelled for each time interval following the cooking step during the Mascarpone production stage. Modelling of *L. monocytogenes* growth commenced at the time when the curd temperature cooled to 40°C after being scooped from the vat.

Mascarpone production (0-4 hr)

The initial four hours of the production process after the cooking stage provide the most favourable conditions for *L. monocytogenes* growth. Growth was modelled after the curd cools to 40°C, and during the subsequent hours, as the curd temperature drops by ~ 30°C. The predicted *L. monocytogenes* growth for this four hour period is shown in Fig 6.2a (mean = log 0.61, 95th percentile = log 0.76), with temperature (c = 0.90) and pH (c = 0.30) being the most important correlation factors (Fig 6.2b). It can be seen from the shape of the predicted growth curve that the conditions present within the curd during the initial portion of the cooling curve are favourable for the proliferation of *L. monocytogenes*, as the curve is strongly weighted towards the higher levels of growth.

Mascarpone production (4-8 hr)

After the initial four hours, the cools to 9.5 ± 2.3°C and the pH is 5.90 ± 0.23. The Mascarpone is moved into the coolroom during this period. It can be seen that the corresponding level of predicted *L. monocytogenes* growth is greatly reduced (Fig 6.3a). The maximum predicted increase is one generation of growth (50th percentile = log 0.09, 95th percentile = log 0.18). Temperature (c = 0.95) and pH (c = 0.25) are still shown to be the major factors correlating with model outcomes (Fig 6.3b). By the end of this time period, the cheese has further cooled to 4.3 ± 1.2°C.

Mascarpone production (8-12 hr)

The cheese curd temperature further decreases during hours 8-12 hr: it is 3.3 ± 0.7°C at the end of the four hour period. The pH value also drops slightly to 5.86 ± 0.24, resulting in the correlation with pH becoming stronger (c = 0.47). Fig 6.4a shows the resultant level of predicted growth is minimal (mean = log 0.035, 95th percentile = log 0.066). Temperature (c = 0.85) still remains the dominant factor controlling growth; salt concentration has little influence (c = 0.03) (Fig 6.4b).

Figure 6.2 - Modelled probability of potential *L. monocytogenes* growth and analysis of sensitivity to input variables during 0-4 hour stage of production

6.2a **6.2b**

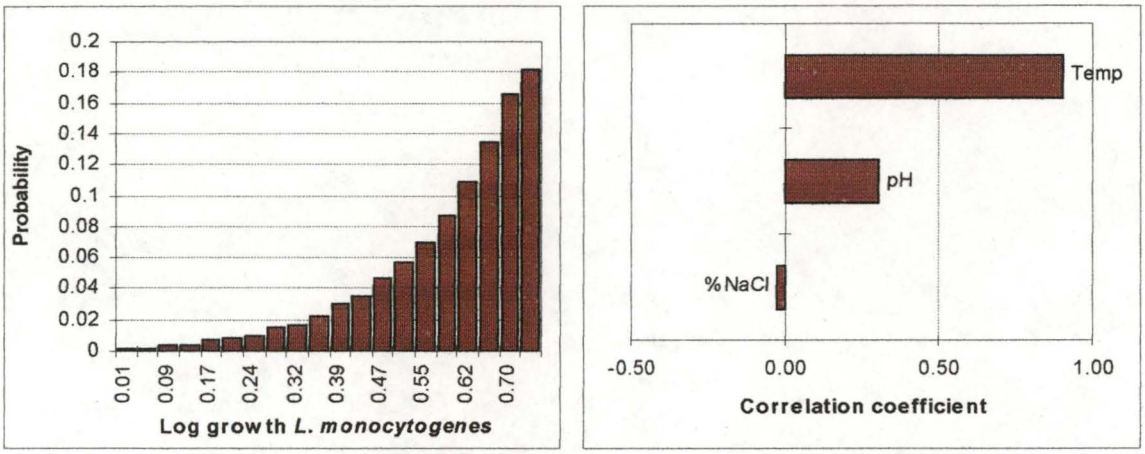


Figure 6.3 - Modelled probability of potential *L. monocytogenes* growth and analysis of sensitivity to input variables during 4-8 hour stage of production

6.3a **6.3b**

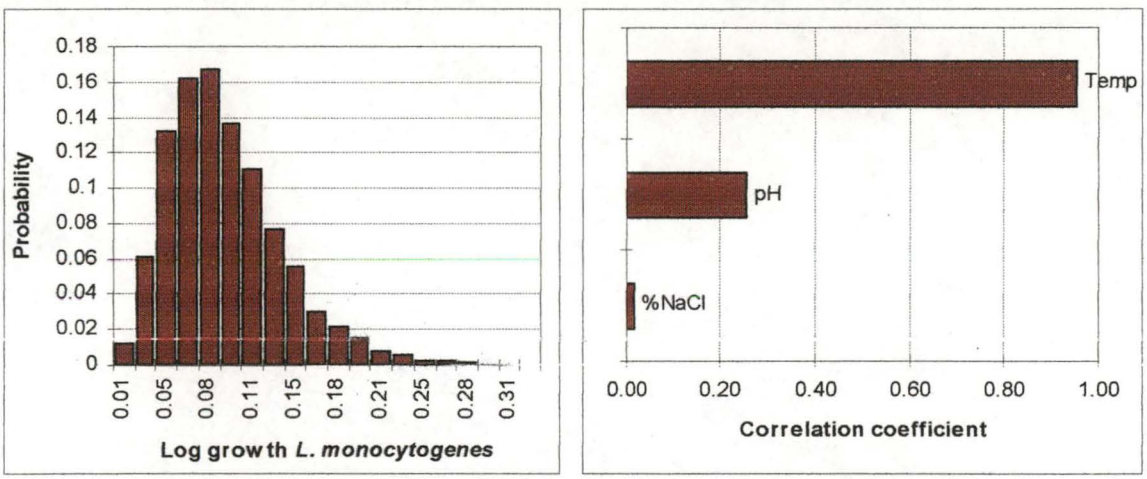


Figure 6.4 - Modelled probability of potential *L. monocytogenes* growth and analysis of sensitivity to input variables during 8-12 hour stage of production

6.4a **6.4b**

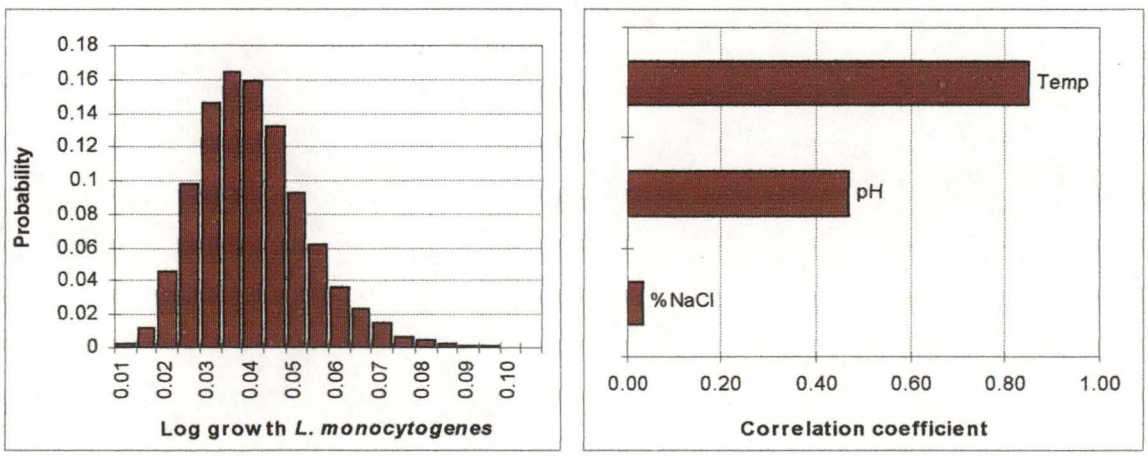


Figure 6.5 - Modelled probability of potential *L. monocytogenes* growth and analysis of sensitivity to input variables during 12-17 hour stage of production

6.5a **6.5b**

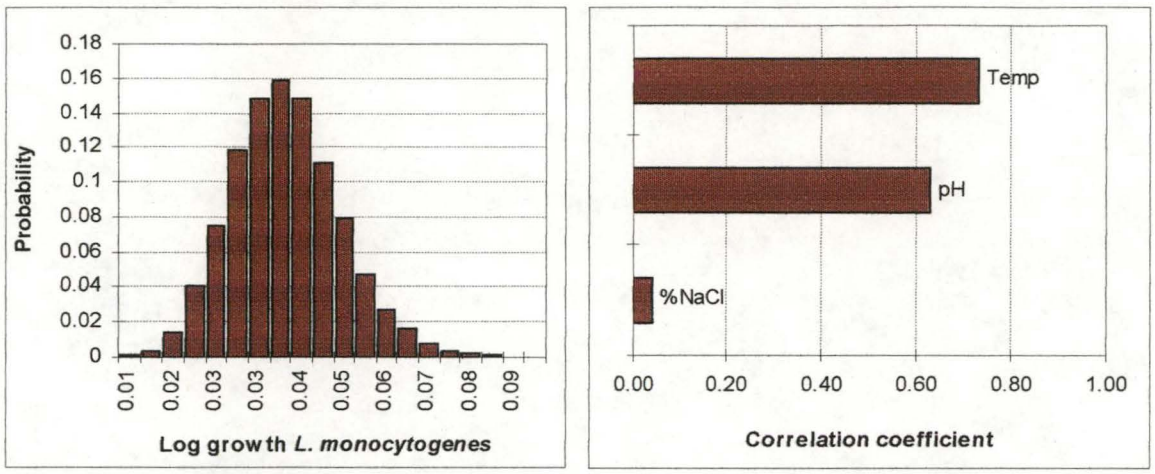
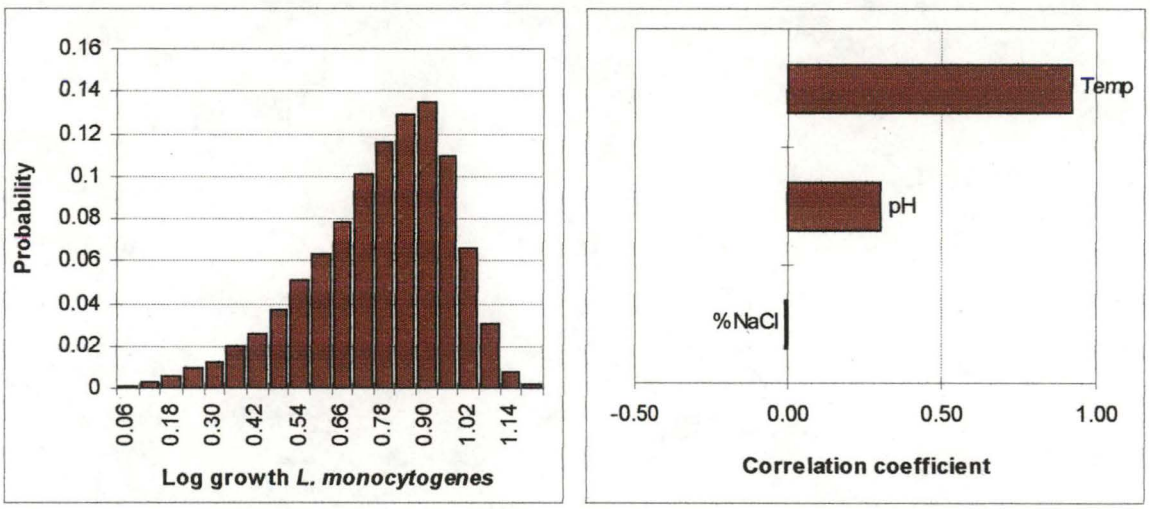


Figure 6.6 - Modelled probability of potential *L. monocytogenes* growth and analysis of sensitivity to input variables during total production stage

6.6a **6.6b**



Mascarpone production (12-17 hr)

In the last five hours prior to packaging, the product temperature does not rise above 3.5°C (Fig 6.1a), while the average pH value remains relatively constant (Fig 6.1b). The amount of predicted *L. monocytogenes* growth is minimal (Fig 6.5a), with a mean increase of log 0.042 (95^{th} percentile = log 0.062). Despite the constant values during this stage, the correlation of model outcomes with pH becomes much stronger than in any of the previous four hour periods (c = 0.63) (Fig 6.5b). Salt concentration still has little correlation with *L. monocytogenes* growth (c = 0.04).

Mascarpone production – totals

The totals from the entire production stage show the most probable increase in the level of *L. monocytogenes* in the product is 2-3 generations (mean = log 0.79, 95th percentile = log 1.06). The majority (~ 75%) of this growth occurs in the first four hours. Temperature was shown to be the most significant factor in controlling growth throughout the entire Production stage (c = 0.92). However, pH (c = 0.30) was also shown to correlate strongly with model outcomes in the latter portion of this stage, despite little change in pH value from earlier in the stage. Salt concentration has little influence throughout the entire production process (c = -0.002).

6.3.2.3 Mascarpone final product attributes

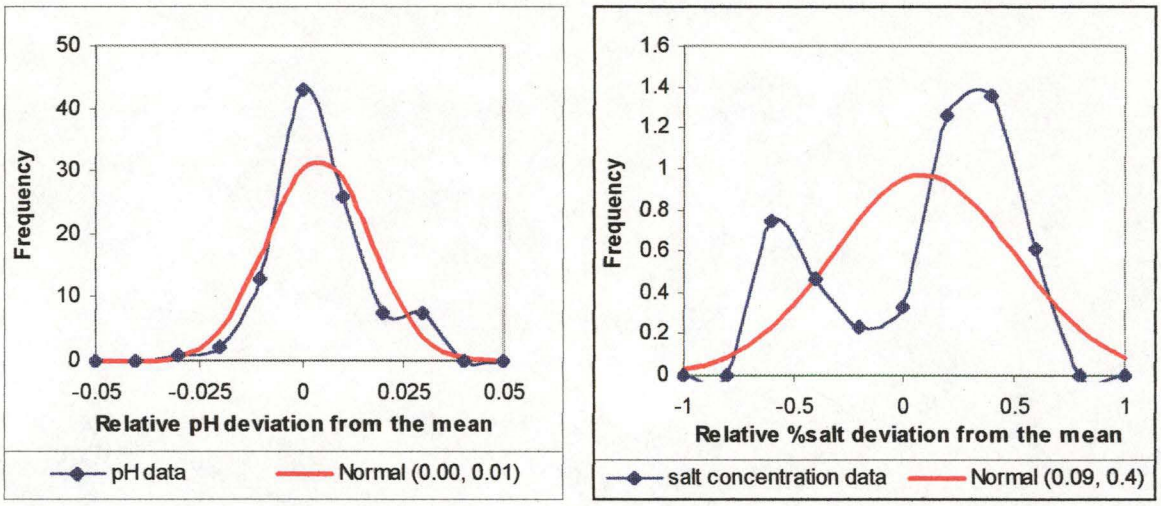
Mascarpone final product attributes are listed in Table 6.5. The average contamination levels found in the final packaged Mascarpone were similar to that found in the Ricotta (Table 5.5), indicating this product is subject to contamination rates similar to those measured in the previous Case Study. The results for standard plate count show that there is a wide range of levels of contamination encountered, with a 3 log difference between minimum and maximum values. The distributions for the final product pH (n = 89) and a_W values (n = 107) are shown in Figs 6.7a-b. As noted in previous plots of calculated salt concentration data, more than one distinct peak was observed (see Section 5.3.2.2).

Table 6.5 - Final product attributes for 1 kg Mascarpone

	pH	a_W	SPC (log cfu/g)	Yeast (log cfu/g)	Mould (log cfu/g)
mean	5.74	0.995	5.98	5.08	4.90
SD	0.07	0.003	0.73	0.59	0.53
High	5.90	0.999	7.02	5.70	5.63
Low	5.55	0.991	4.18	4.32	4.53

Figure 6.7 - Mascarpone final-product pH and calculated salt concentration and Comparison with Normal distribution selected by BestFit software

6.7a **6.7b**



As in other Case Studies, ten cheese samples were tested for the presence of pathogenic microorganisms using methods detailed in Chapter 2 (Section 2.2.3).

6.3.2.4 Mascarpone Storage and Distribution

The Mascarpone was also manufactured by Factory 'B', therefore the coolroom storage and distribution data were exactly the same as used in the previous Ricotta Product Case Study.

6.3.2.5 Mascarpone Shelf life

There were no significant differences in the changes observed for pH and a_W during the cheese shelf life, regardless of storage temperature. Therefore, the data for both storage temperatures were collated and the parameter values and distributions determined collectively. Fig 6.8 shows the pH development during the cheese shelf life and the normal distribution used to describe the model input The changes in calculated salt concentration are shown in Fig 6.9. Therefore, the same parameter values and distributions were used for modelling predicted *L. monocytogenes* growth during the shelf life at both 5°C and 10°C.

The model outcomes for shelf life storage at 5°C (Fig 6.10a) and 10°C (Fig 6.11a) show the marked difference in predicted growth under those storage

conditions. The higher storage temperature allows 3-times more growth to occur, under identical conditions of pH and a_w. As in previous Case Studies, the predicted theoretical generations of growth exceeds what would be observed in reality. Therefore, an upper limit of 10^8 cfu/g was specified in the final risk assessment model. The sensitivity analyses demonstrate the dependence these outcomes have on the temperature. At 5°C (Fig 6.10b), the model outcomes are almost totally correlated to temperature (c = 0.98), with pH exerting moderate influence (c = 0.16) and salt concentration very little (c = 0.03). The sensitivity analysis for storage at 10°C (Fig 6.11b) is virtually identical.

Figure 6.8 - Mean pH during Mascarpone shelf life (—), upper and lower limits (—) and Comparison with Normal distribution selected by BestFit software

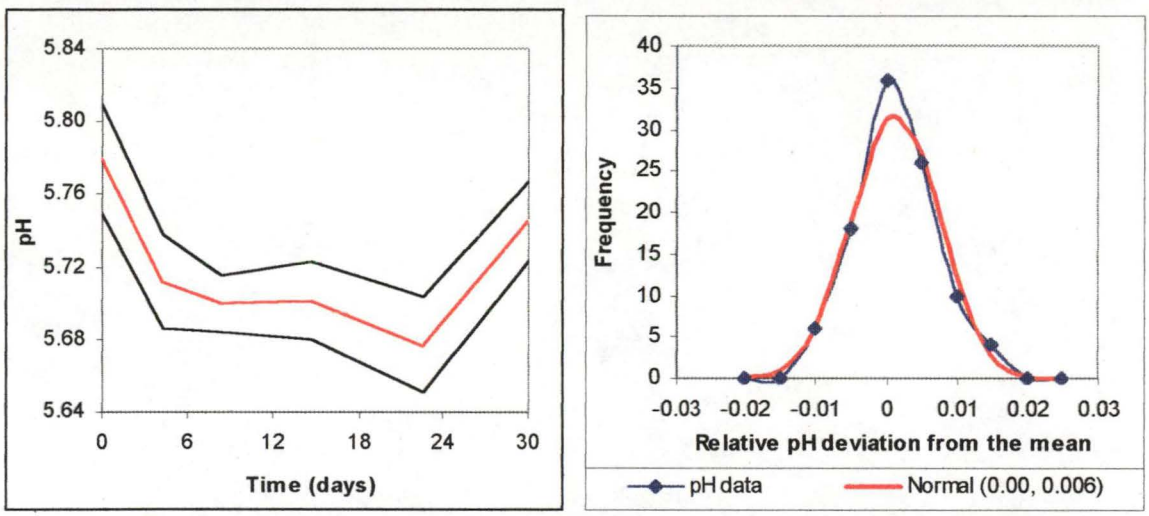


Figure 6.9 - Mean calculated salt concentration during Mascarpone shelf life (—), upper and lower limits (—) and Comparison with Normal distribution

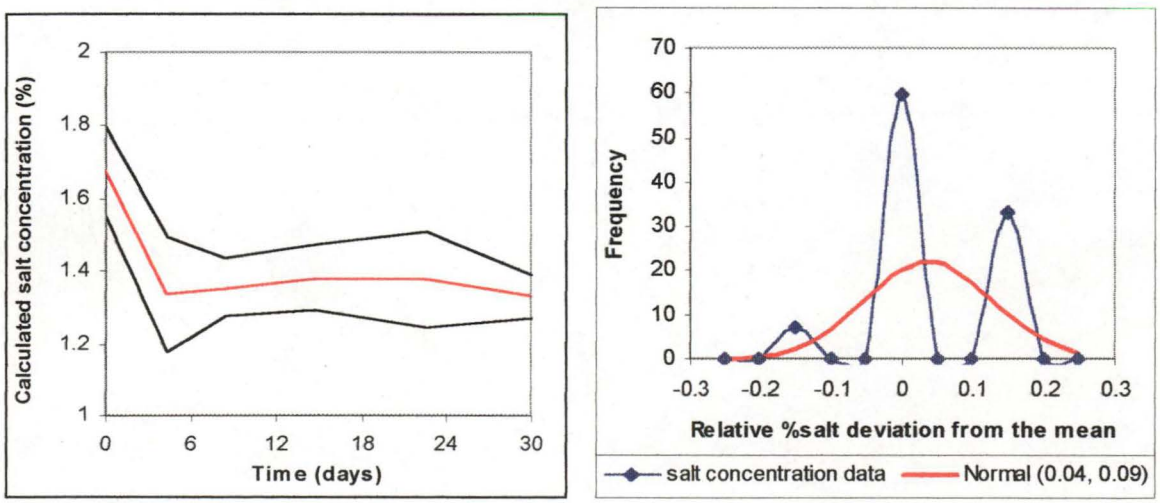


Figure 6.10 - Modelled probability of potential *L. monocytogenes* growth during Mascarpone shelf life at 5°C and analysis of sensitivity to input variables

6.10a **6.10b**

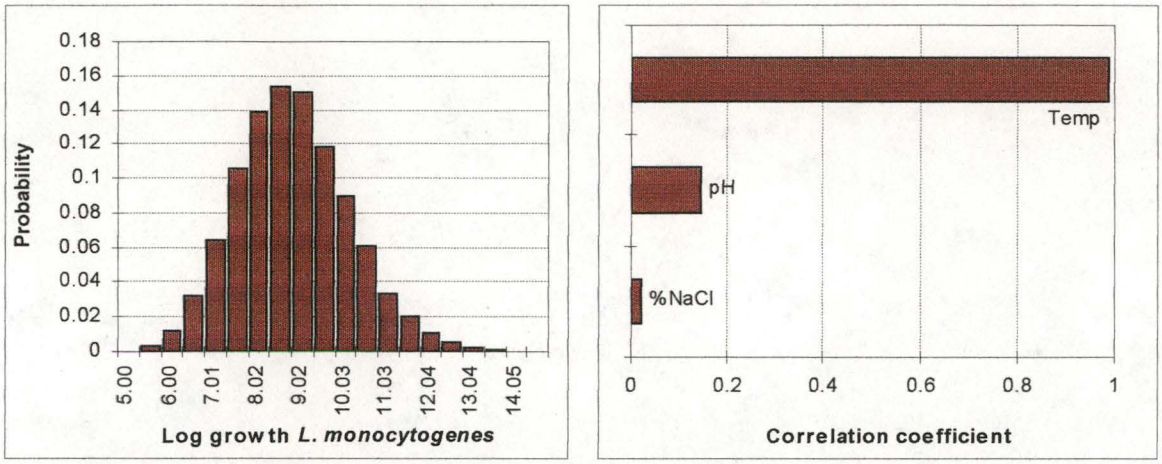
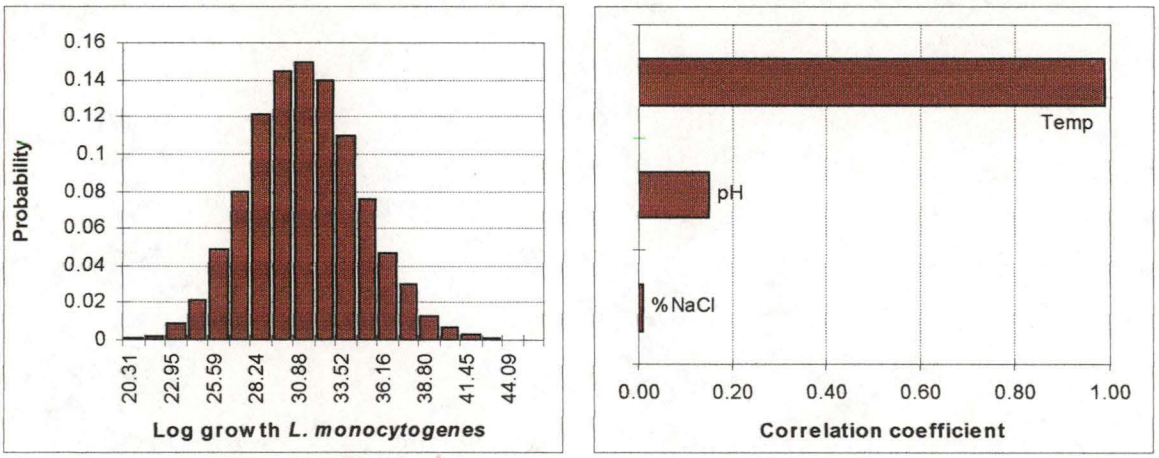


Figure 6.11 - Modelled probability of potential *L. monocytogenes* growth and analysis of sensitivity to input variables during Mascarpone shelf life at 10°C

6.11a **6.11b**



Microbiological profiling of the shelf life showed that bacterial numbers remain relatively constant for the duration of the 28 days, regardless of whether the cheese was stored at 5°C (Fig 6.12) or 10°C (Fig 6.13). The standard plate count slowly climbed, mirrored by a slight increase in the number of (non-starter) lactic acid bacteria. The number of yeasts and moulds did not vary considerably, remaining at a level of ~10^2-10^3 cfu/g for the entire storage period. There appeared to be no significant difference in microorganism levels between the two storage temperatures, which may explain the constancy of pH values also remaining constant at both storage temperatures.

Figure 6.12 - Microbiological profile of 1kg Mascarpone during shelf life storage at 5°C

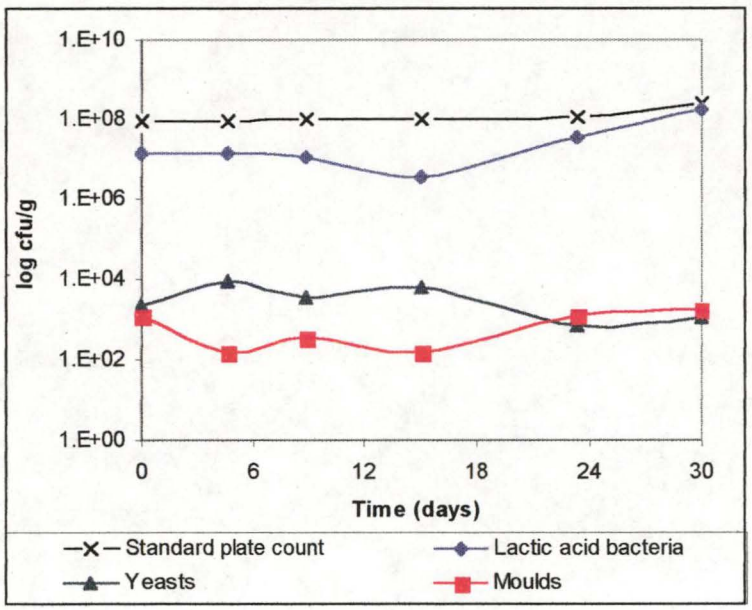
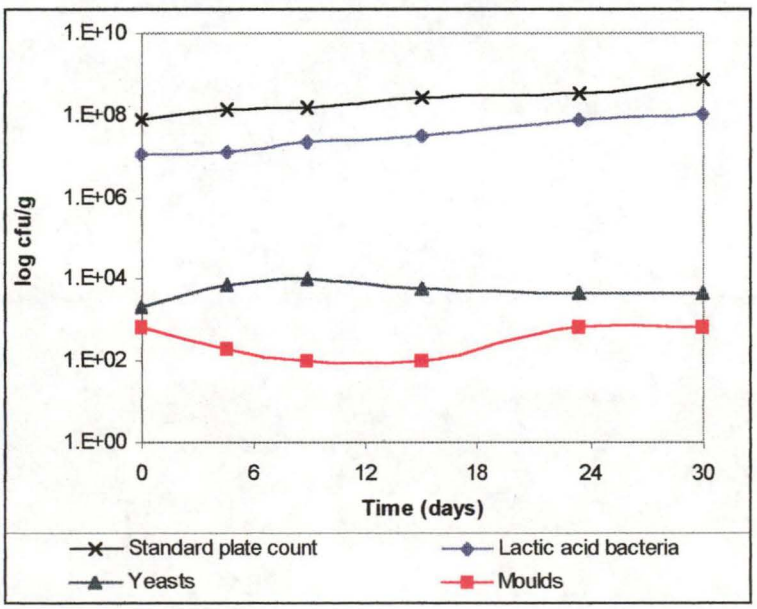


Figure 6.13 - Microbiological profile of 1kg Mascarpone during shelf life storage at 10°C



6.3.3 Mascarpone detailed Quantitative risk assessment

6.3.3.1 Time of contamination

The Mascarpone manufacturing process is susceptible to airborne contamination during the cooling phase of the process. The microbiological profile (Fig 6.14) suggests that it is the initial few hours after the cooking process, while the curd is cooling that most contamination occurs. Some of the observed increase in numbers may also be due to heat-injured cells recovering, or the germination of spore-formers. The product would be subject to similar contamination rates to those which were measured in the previous Case Study, except that the time the product spends in the production room areas is shorter.

The Mascarpone curd is also covered at a much earlier stage than the Ricotta, however there did not appear to be less contamination in the final product. Therefore, as was concluded in the previous Case Study, although the rate of contamination may differ during different stages, it was considered that the probability of *L. monocytogenes* contamination was uniform throughout the process, and a uniform distribution was specified for the risk assessment model input parameter (Table 6.1).

6.3.3.2 Number of serves

The estimated number of serves of Factory 'B' Mascarpone in Tasmania *per annum* is shown in Fig 6.15. The mean predicted number of serves was 40,942 (50^{th} percentile = 32,661, 95^{th} percentile = 93,727). Therefore, although the production level of this cheese is only 1.2 tonnes per year, it is consumed on more than forty thousand occasions. It therefore has the potential to infect a large number of people should it become contaminated.

6.3.3.3 Level of L. monocytogenes at end of storage and distribution

Similar to the corresponding outcome from the Ricotta Case Study (Chapter 5), the model predictions for the level of *L. monocytogenes* at the end of storage and distribution (Fig 6.16) show a distribution almost identical to the 'Contamination level' Triangular input. There is even less of a shift observed in the model outcomes than was observed in the Ricotta model, with the mean level of *L. monocytogenes* at the end of Storage and Distribution log –0.32, and the maximum log 3.27. The sensitivity analysis (Fig 6.17) demonstrates the strength of the correlation between model outputs and the initial contamination level (c = 0.998). This tends to indicate that, on average, little growth occurs during the production and storage stages.

Figure 6.14 - Typical microbiological profile of Mascarpone manufacture

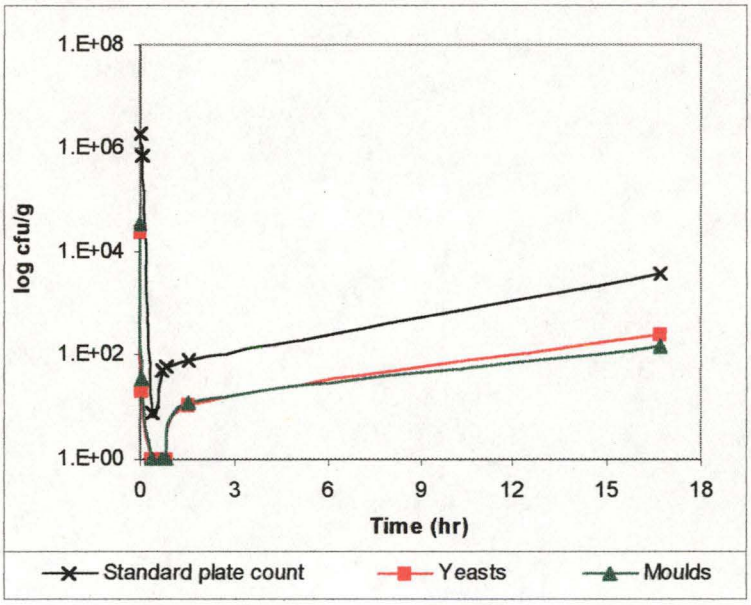


Figure 6.15 – Number of Mascarpone servings *per annum* in state of Tasmania

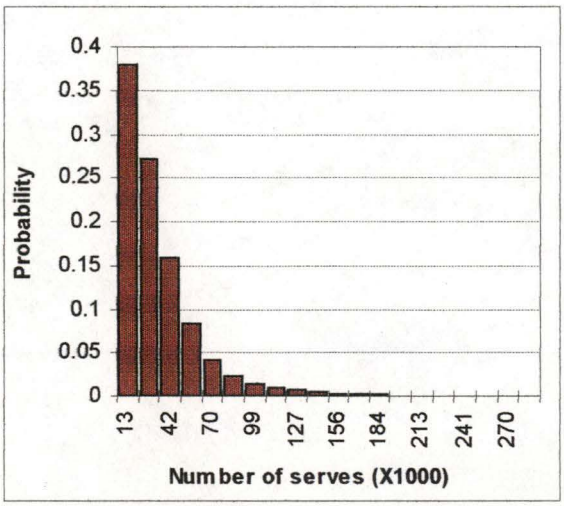


Figure 6.16 – Predicted level of *L. monocytogenes* at end of storage and distribution stage for contaminated Mascarpone cheese

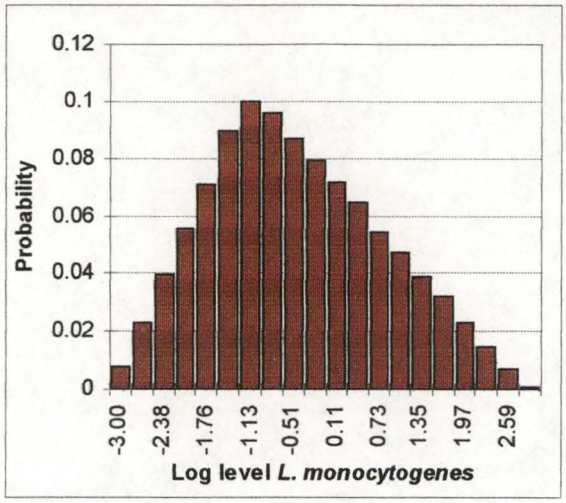
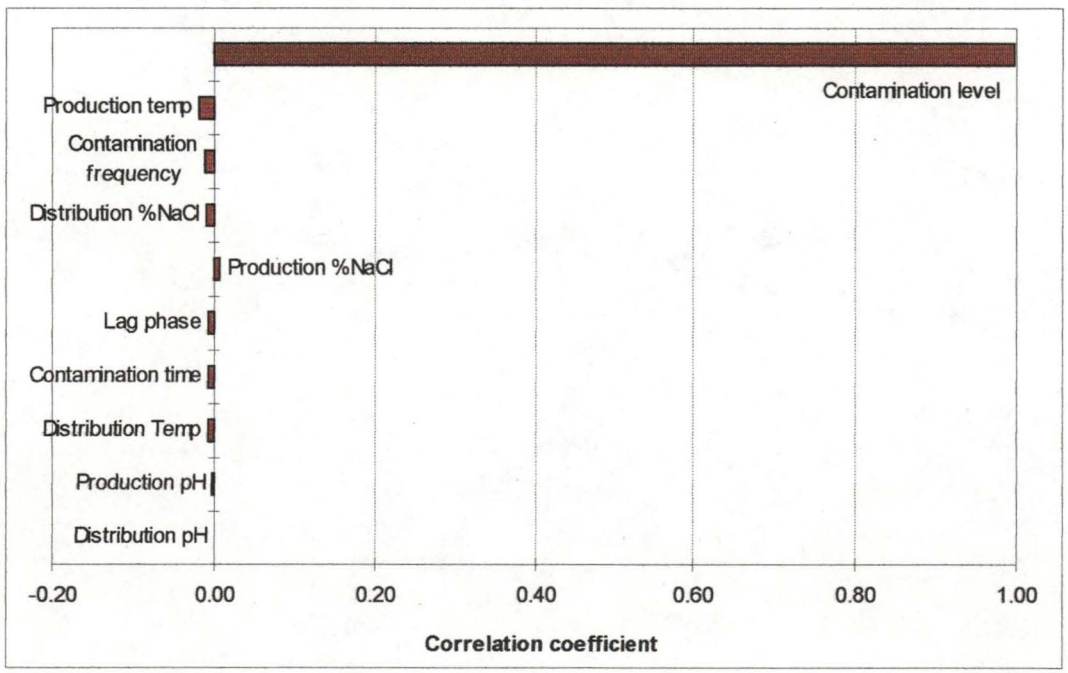


Figure 6.17 – Sensitivity analysis of model inputs for predicted level of *L. monocytogenes* in Mascarpone cheese at end of storage and distribution



The Process Risk Model demonstrated that approximately 75% of growth in the production stage occurs in the initial four hours. Any *L. monocytogenes* contamination that occurs after this point will be subject to unfavourable temperature conditions, and contamination that does occur in the first four hours will most likely remain in lag phase for a portion of time when the conditions are most favourable. As in the Ricotta risk assessment (Chapter 5), the sensitivity analysis does not show a strong correlation existing between model outcomes with either contamination time or lag phase. Production temperature was shown to have the second highest correlation factor, however it was very small (c = -0.018).

6.3.3.4 Level of L. monocytogenes at time of consumption

The model predicted a large increase in numbers of *L. monocytogenes* to occur between the end of storage and distribution, and the time when the cheese is consumed. Fig 6.18 shows the range of *L. monocytogenes* levels predicted in the cheese at the time when it is consumed (mean = log 6.39, 50th percentile = log 2.11, 95th percentile = log 6.58). The mean level is skewed upwards by the higher levels, therefore it is more meaningful to consider the 50th percentile level rather than the mean. This shows that approximately fifty percent of cheese samples will contain less than 100 *L. monocytogenes* cells/g.

The analysis of sensitivities (Fig 6.19) is able to clearly demonstrate the factors predicted to most affect *L. monocytogenes* growth. As in previous Case Studies, the time when the cheese is consumed is a major determinant of the final level of *L. monocytogenes*, and as a result, the risk posed by the cheese. It was shown in Section 6.3.2.5 that *L. monocytogenes* was able to grow to high levels during the course of the shelf life, therefore the earlier the cheese is consumed, the less *L. monocytogenes* growth will be able to occur. The other major factors correlating with *L. monocytogenes* growth are lag phase, production temperature, and contamination level.

6.3.3.5 Dose of L. monocytogenes

The dose of *L. monocytogenes* present on the cheese at the time of consumption is shown in Fig 6.20. The dose level ranges from one cell per 50 g cheese, through to 1.15 x 10^{10} bacterial cells (50th percentile = 6.1 x 10^{3}, 95th percentile = 1.84 x 10^{8}). The sensitivity analysis (not shown) for Dose is almost identical to Fig 6.19.

Figure 6.18 – Predicted level of *L. monocytogenes* at time of consumption for contaminated Mascarpone cheese

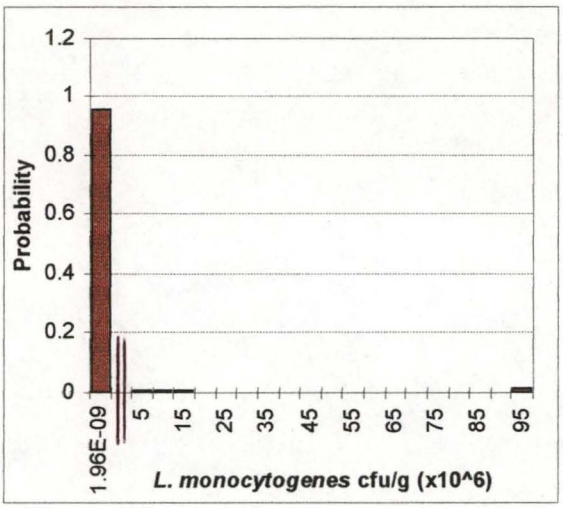


Figure 6.19 – Sensitivity analysis of model inputs for predicted level of *L. monocytogenes* in Mascarpone cheese at time of consumption

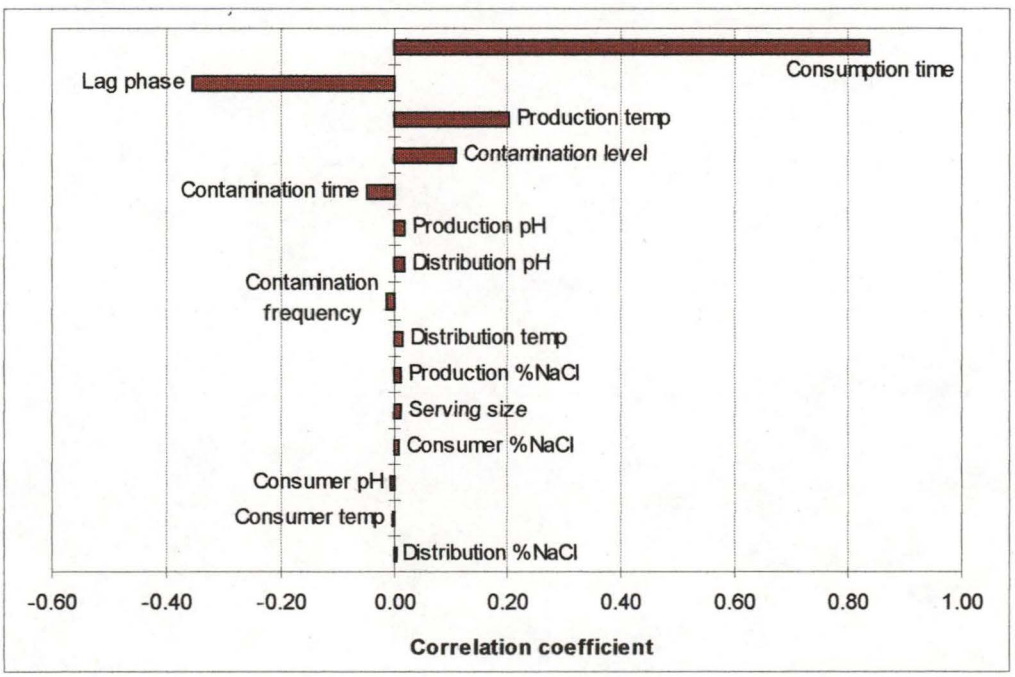
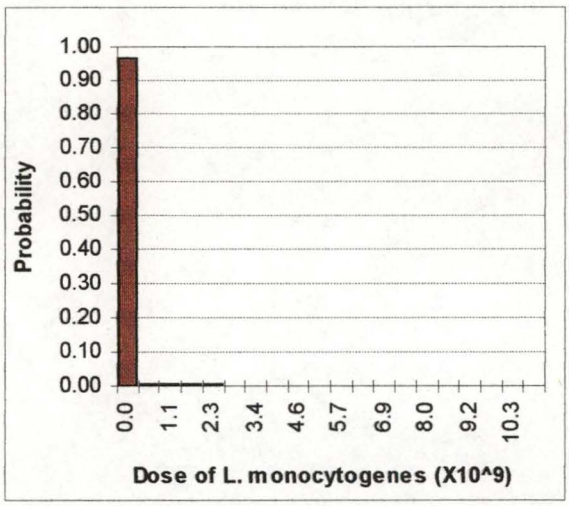


Figure 6.20 - Predicted dose of *L. monocytogenes* on a serving of cheese.



6.3.3.6 Probability of infection per meal

The probability of infection from contaminated Mascarpone was calculated from the R-value of Ross (*unpublished*), and was found to range from 0 to 2.14×10^{-4} (mean value = 2.35×10^{-6}). Unlike the previous Case Studies (Chapters 4 and 5), the minimum probability of infection from the consumption of Mascarpone was calculated as being zero (despite the value being calculated as probability from consumption of contaminated cheese only). A minimum probability of zero was possible since the minimum calculated dose was equivalent to one cell per 50g, therefore a smaller serving size than this would results in no *L. monocytogenes* cells being ingested.

6.3.3.7 Predicted number of listeriosis cases per annum

The number of predicted listeriosis cases from the consumption of Factory 'B' Mascarpone was very low both for the general population (Fig 6.21a) and the susceptible population groups (Fig 6.21b), with the results summarised in Table 6.7. The mean number of total cases in a year was calculated to be 0.0035, which would result in one listeriosis case in 285 years. However, the maximum outcomes from the model predicted a total of 0.34 cases per year, or one listeriosis case every 3 years. This was predicted to be a very rare outcome based on the 95th percentile value.

The sensitivity analysis (Fig 6.22) was found to be very similar to that determining the level of *L. monocytogenes* at the time of consumption.

Figure 6.21 – Distribution of Listeriosis cases per annum in Tasmania as a result of Mascarpone consumption a) general population; b) susceptible population Contamination frequency Triangular (0, 0.003, 0.043)

6.21a **6.21b**

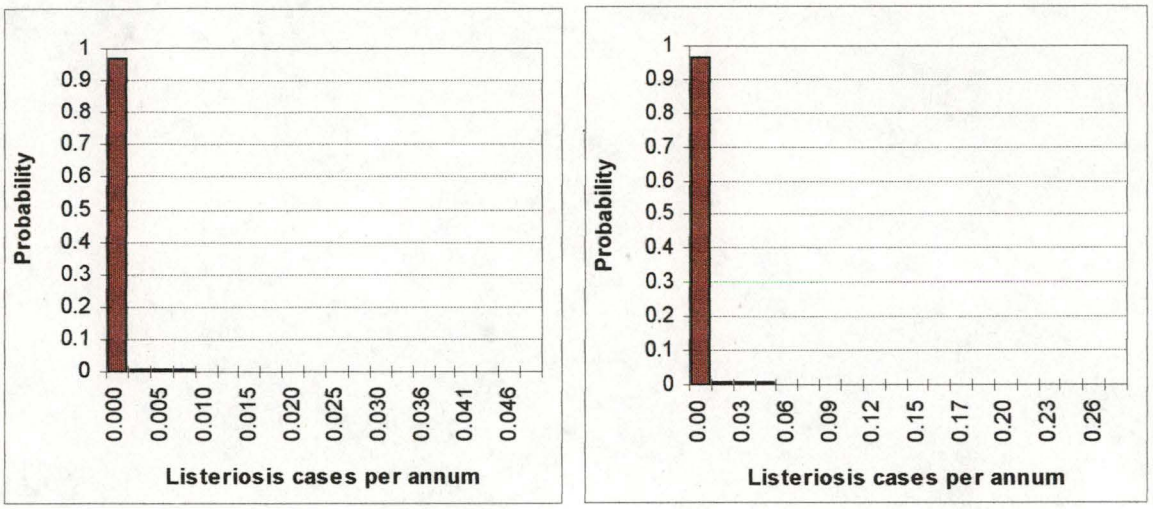


Figure 6.22 – Sensitivity analysis for model inputs for risk of listeriosis per annum

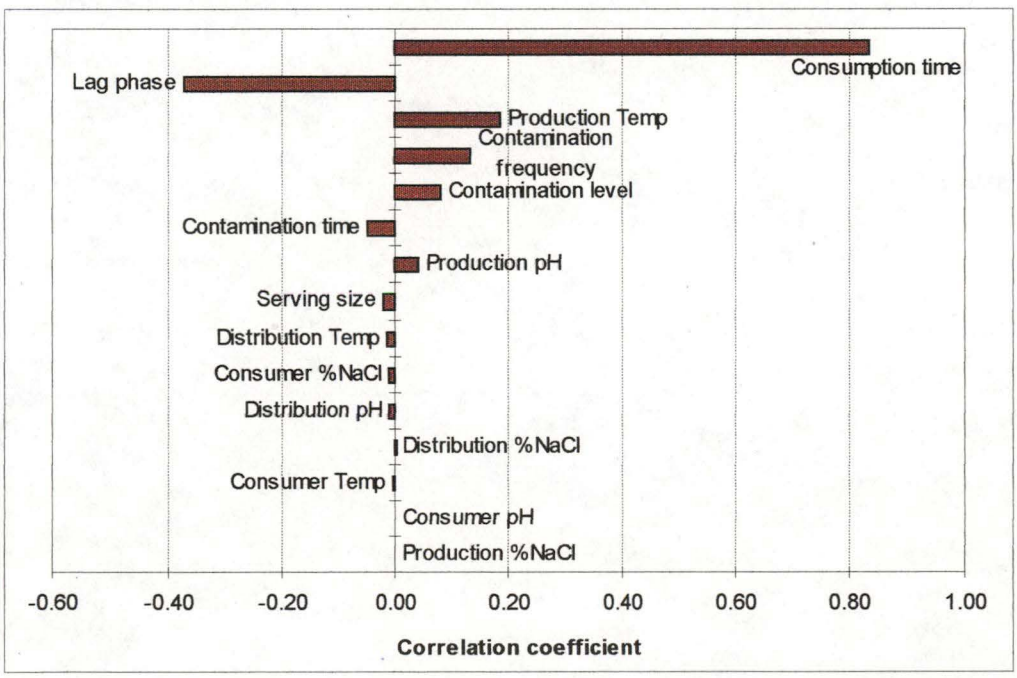


Table 6.7 – Predicted number of listeriosis cases per annum predicted from consumption of Factory 'B' Mascarpone in Tasmania, based on age and susceptibility

Tasmania					
	Minimum	**Mean**	**Maximum**	**50th percentile**	**95th percentile**
Cancer	0	1.22e-4	1.22e-2	5.87e-9	1.83e-4
Transplant	0	3.99e-4	3.85e-2	1.85e-8	5.77e-4
AIDS	0	3.15e-4	3.05e-2	1.46e-8	4.57e-4
Diabetes	0	6.56e-4	6.35e-2	3.05e-8	9.50e-4
Pregnant	0	8.44e-4	8.18e-2	3.92e-8	1.22e-3
Kidney	0	2.44e-5	2.36e-3	1.13e-9	3.54e-5
> 60 years	0	5.43e-4	5.26e-2	2.52e-8	7.87e-4
< 30 days	0	9.22e-5	8.93e-3	4.28e-9	1.34e-4
Susceptible population Total	**0**	**3.00e-3**	**0.290**	**1.39e-7**	**4.34e-3**
1 – 9 years	0	1.74e-5	1.68e-3	8.08e-10	2.52e-5
10 – 19 years	0	2.02e-5	1.96e-3	9.39e-10	2.93e-5
20 – 29 years	0	6.52e-5	6.32e-3	3.03e-9	9.45e-5
30 – 39 years	0	1.84e-4	1.78e-2	8.54e-9	2.66e-4
40 – 49 years	0	9.78e-5	9.47e-3	4.55e-9	1.42e-4
50 – 59 years	0	1.39e-4	1.35e-2	6.47e-9	2.02e-4
General population total	**0**	**5.24e-4**	**5.07e-2**	**2.43e-8**	**7.59e-4**

6.3.3.8 Alteration of input parameter estimates

Possible risk mitigation strategies were evaluated for their effectiveness in lowering the risk of listeriosis from consumption of Factory 'B' Mascarpone. These strategies were implemented in the model by altering input parameter estimates.

Effect of storage temperature

The cheese storage temperature during consumer handling was increased in the model to 10 ± 0.5°C to gauge the effect on listeriosis risk. The dose of *L. monocytogenes* at the time of consumption was predicted in the range log 0.156 to 1.23×10^{10}. The mean probability of infection increased by only 7% to 2.30×10^{-4}, with the number of listeriosis cases predicted to increase by a similar amount.

Effect of limiting shelf life

The model assumptions were tested by limiting the shelf life by one week to 21 days. The mean probability of infection per meal was reduced to 1.13×10^{-4}, an almost 50% decrease on the initial model outcomes. This was reflected in the corresponding number of predicted listeriosis infections within the consuming population, with a mean predicted number of 6.69×10^{-5} cases per year, and a maximum number of 0.15. All results are presented in Appendix G.

6.4 Discussion

There is very little published information regarding Mascarpone cheese, and no published growth data regarding the behaviour of *L. monocytogenes* in the cheese to draw comparison with. Despite the product having the lowest final pH of any of the three products considered in this study, the PRM showed that growth of *L. monocytogenes* to high levels is still possible. The initial hours of processing, while the curd is cooling, contribute approximately 75% of the predicted growth for the entire production stage. Therefore, as was detailed in the Ricotta Case study, a risk mitigation strategy could be to ensure that the curd cools as rapidly as possible.

It was shown in the detailed risk assessment results that the risk of contracting listeriosis from Mascarpone is the lowest of the three cheese products studied here. The mean calculated probability (mean = 2.35×10^{-6}) places Mascarpone at less than half the risk of Ricotta (mean = 5.28×10^{-6}), and 30 times safer than Brie (mean = 7.17×10^{-5}). There are a number of factors which can be used to explain this. The most obvious being that there is only 1.2 tonnes of Factory 'B' Mascarpone consumed *per*

annum as compared to 4 tonnes of Factory 'B' Ricotta and 125 tonnes of Factory 'A' Brie. Therefore, on a consumption quantity basis, this indicates that the *risk per serving of cheese* is actually higher for Mascarpone, than the other two cheese products.

Mascarpone cheese has the lowest pH value of all three cheeses. Despite the acidity not being at a sufficient level to inactivate *L. monocytogenes*, it still retards the growth to a degree and provides another factor besides storage temperature which can be used to inhibit the growth of *L. monocytogenes*. The Murphy-model, used in this thesis for predicting the growth of *L. monocytogenes,* bases its predictions solely on the effect of pH on *L. monocytogenes* growth. It does not account for the inhibitory effects of dissociated organic acids. It has been determined that organic acids differ in their inhibitory properties, based on their pK_a value, and the resultant proportion of associated and undissociated acid. This effect has been mainly investigated for lactic acid, and the effect modelled for *Listeria* by El-Shenawy & Marth (1989), George *et al.* (1996), and Tienungoon *et al.* (2000). The relative effect of undissociated organic acids has also been described for *Yersinia* (Adams *et al.*, 1991) and *E. coli* (Presser *et al.,* 1998). This becomes relevant when comparing predictions of *L. monocytogenes* growth on cheeses using different acidulants. Tartaric acid and citric acid are added directly to coagulate the curd in forming Mascarpone and Ricotta, and the manufacture of Brie is dependent upon production of lactic acid by starter cultures.

The acidification step within Mascarpone production was not classified as a CCP within the Food Safety Scheme prepared for the factory. Since the acidity is not low enough to inactivate *L. monocytogenes*, this was correct according to the true definition of a CCP. However, it is important, that the acidification step should be captured within the GMP programs which support the HACCP plan. Insufficient acidification of the cream will not only lead to poor coagulation and resulting poor yield (Table 6.4), but lead to a product where *L. monocytogenes* will be potentially able to grow at a faster rate, thus increasing the risk of the final product. Although not covered in this study, the stochastic model could easily be adjusted to investigate the effect of final product on the risk of listeriosis. However, from the sensitivity analysis shown in Fig 6.22, product pH was shown not to correlate highly with cases of listeriosis.

The risk assessment model outcomes, and the results of the challenge tests from Chapter 3, demonstrate that *L. monocytogenes* is capable of reaching high levels within the product during the course of the shelf life. The model also showed that *L. monocytogenes* growth is possible during all stages of the manufacturing process, therefore the only Critical Control Point lies with the heat treatment of the raw cream. Within the Food Safety Scheme developed for the factory, the points of Cream receival and cream storage were classified as Critical Control Points (Tables 6.3 & 6.4). However, as was discussed in the Ricotta Case Study (Chapter 5), any microbial growth which can potentially take place prior to the heat treatment would be inactivated by the harshness of the cooking step. This renders the designation of these steps as CCPs as ineffective, although control of cream quality and storage temperature are important for the palatability of the end product (Table 6.4). Although Aseptic packaging and GMP are imperative in minimising the risk of recontamination with *L. monocytogenes.*

The risk mitigation strategies presented in previous Case Studies such as control of consumer storage temperature were not as effective in reducing the risk of listeriosis from Mascarpone as with the other products studied in this thesis. However, the time of consumption was shown to most highly correlate with risk of listeriosis, and it was shown how drastically the risk could be decreased by limiting the shelf to 3 weeks, rather than 4 weeks (a 50% decrease in risk). Since Mascarpone appears to be eaten relatively quickly, it may be a risk mitigation strategy for the factory to limit the shelf life of the product to less than 21 days. As with the previous Case Studies, the results of the model must be interpreted with care as the dependency of the model outcomes on the assumed parameters was once again highlighted.

6.4.1 Conclusions

The detailed risk assessment presented in this Case Study has shown that, due to the low levels of Mascarpone consumption, the probability of this product causing a case of listeriosis is the lowest of all three cheese products studied in this thesis. However, on a *per serving* basis, then Mascarpone was shown to present a higher risk than both Brie and Ricotta.

The isolation of high levels of *H. alvei* (Appendix H), while not directly related to the risk of listeriosis, does demonstrate the potential for contamination to occur within this cheese and that growth to a high level is possible. This incident however,

did serve to highlight the need for vigilance in preventing contamination during the manufacture of Mascarpone. It is extremely unlikely that the high level of bacteria recovered from the product was contributed by a single contamination event and most likely resulted from growth of the organism following contamination. The risk assessment model outcomes showed that high levels of *L. monocytogenes* could occur on the cheese should several factors coincide for this to take place. Assuming temperature control is maintained throughout the process, the majority of growth occurs within the first four hours of the production stage, and then during the shelf life of the cheese. The contamination with *H. alvei* has shown that these risk assessment results may be applicable to other contaminating psychrotrophic microorganisms.

7. Summary and Conclusions

Notermans & Jouve (1995) stated that regulatory authorities, and by inference the general population, have to accept that completely safe food does not exist. The original intention of Quantitative Microbial Risk Assessment was to provide an estimate of how far from 'absolutely safe' the food was, in terms of the probability and impact of an adverse health outcome due to a microbial hazard. However, since its initial inception, there has been a shift in the thinking towards where QMRA may be applied. Cassin *et al.* (1998b) suggested that a higher priority goal of QMRA may be to provide information to support decisions regarding risk mitigation strategies. Miles & Ross (1999) supported the approach that QMRA will most commonly be used in the future for identifying and ranking steps that contribute risk.

This thesis has emphasised the lack of data needed to complete many aspects of the risk assessment process, and the resultant number of assumptions required for the application of QMRA. Where estimates have been necessary in the present calculations, these have been highlighted to give the assessment transparency. Two approaches have been utilised in this thesis to generate outcomes with different goals in mind. A simple risk assessment model was formulated to identify steps within cheesemaking, distribution and storage processes that allow significant growth of *L. monocytogenes,* and to pinpoint the significant growth-controlling factors. Reduction in the temperature, pH and a_W of the cheeses can limit the extent to which the organism will grow, however, it has been demonstrated in the Case Studies that the potential exists in some cases for *L. monocytogenes* to reach high population numbers prior to consumption. Furthermore, through the development of a detailed risk assessment, it has been demonstrated in this thesis, that it is possible to develop a realistic estimate or, at the very least, a semi-quantitative evaluation of the risk involved with the consumption of a particular cheese product.

It has been shown in the process analysis, that the sole Critical Control Point in the production of Brie, Ricotta and Mascarpone is the heat treatment or cooking process. The use of hygiene and sanitation as complementary support programs to HACCP is a necessity to ensure post-pasteurisation contamination does not occur. This is in agreement with D'Aoust (1989), who suggested the mandatory use of pasteurised milk may provide the only viable option for the production of pathogen-free dairy products. No other steps within the cheesemaking process will reduce the level of *L. monocytogenes* in these products. However, strategies were presented,

such as limiting the cheese shelf life, which were shown to significantly reduce the listeriosis risk. Education of susceptible consumers to avoid these products or to take special precautions in their handling of it, may be an added strategy to prevent listeriosis cases from these products.

Any modelling system is limited in its applicability, in that it attempts to simplify a complex system. Therefore limitations of the model must be kept in mind and the model outcomes interpreted with caution. However, a major advantage of defining the cheesemaking process in terms of the three modelled parameters (temperature, pH and salt concentration) is that it can allow "real-time" monitoring, allowing the degree of control at a CCP to be determined almost instantaneously. However, over-simplification of the process into parameters of temperature, pH and water activity may overlook significant interactions among other factors, for example the interaction between pH and organic acid levels. The ability to monitor the microbiological safety of a process with rapid measurements, which in many cases are already carried out by processors, would decrease the need for much of the microbiological testing which is currently conducted.

Despite the limitations of the risk assessment process, the value of the stochastic modelling approach for determining the efficacy of various management options in controlling the risk of listeriosis was demonstrated in this thesis. The predicted number of listeriosis cases presented in this study support the conclusions of previous publications (Farber *et al.*, 1996a; Buchanan *et al.*, 1997a; Notermans *et al.,* 1998) that listeriosis is a rare disease of humans, despite relatively frequent exposure to the organism. Risk assessment provides a quantitative, process–oriented approach to provide or identify the information required to ensure optimal decision-making on pathogen risk reduction strategies. In the future, the aim of both processors and regulators should be to develop risk-mitigation strategies based upon a quantitative appraisal of the advantages and disadvantages of any risk-mitigation options.

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Appendix A – Equipment and Computer software

A.1 - Equipment

pH meter

Orion pH meter 250A (Orion Research Inc. Boston, MA 02129, USA) with Activon AEP 433 flat tip probe (Activon Scientific Products Co. Pty. Ltd., 2A Pioneer Ave, Thornleigh, PO Box 505, Pennant Hills, NSW, Australia, 2120)

Water activity meter

Aqualab CX-2, Decagon Devices Inc., PO Box 835, Pullman, Washington 99163, USA.
Calibrated with distilled water and saturated salt solution before use.

Stomacher and blender bags

Colworth, Stomacher 400, Model BA6021, Single Phase, A.J. Seward, UAC House, Blackfriars Road, London, SE1 9UG used with Bio-Service Pty. Ltd blender bags, PO Box 180, Huntingdale 3166, Australia

Pipettes

A range of fixed and variable volume pipettes were used:
Fixopet: 100μL (fixed), 1000 μL (fixed); Pluripet: 200-1000 μL, Kartell Spa Via, Delle Industrie, 1 20082 Noviglio, Milan, Italy.
Pipetman®: 200-1000μL, Gilson Medical Electronics (France) S.A., B.P. 45-95400, Villiers-le-Bel, France
Oxford Macro-set: 5-10mL, Oxford Labware, Division of Sherwood Medical, St Louis, MO 63103, USA

Centrifuges

'Easyspin' bench-top centrifuge, Sorvall® Instruments DuPont Company, Biotechnology Systems Division, Wilmington, DE 19898, USA
Beckman J2-21 M/E Centrifuge, Beckman Instruments Inc., Spinco Division, 1050 Page Mill Road, Palo Alto, CA 94304, USA

Temperature data loggers

Hastings Data Loggers, 1/8-12 Acacia Avenue Port Macquarie, NSW 2444, Australia
Temperature range -40°C to 120°C

Chamber Vacuum Packing Machine and barrier bags

BUSCH type 100-132, Boss 6380 Bad Homburg 6, West Germany, vacuum 0.5 mbar, motor oil type SAE 30, Timer: second (manual) or automatic used with Cryovac barrier B471 specification bags, Cryovac Division, W.R. Grace Australia Ltd, 1126-1134 Sydney road, Fawkner, Victoria, 3060

Barrier bag specifications: Thickness: 60μm, Oxygen permeability 50 cc/24 hr/m^2/atm at 23°C maximum

Temperature gradient incubator

Advantec TN-2148, Advantec MFS Inc., 6691 Owens Drv, Pleasanton, CA 94588, USA – used with L-shaped glass tubes, 1.5 cm diameter, capacity approximately 25 mL.

Waterbaths

Ratek SWB20D shaking waterbaths, Ratek Instruments Pty Ltd, Unit 1/3 Wadhurst Drv, Boronia, VIC, Australia, 3155.

Lauda RC20 and RM20 non-shaking refrigerated waterbaths

Spectrophotometer

Spectronic 20, Spectronic Instruments, Inc., 820 Linden Ave, Rochester, NY 14625, USA.

Thermometer

Fluke® 51K/J (John Fluke Mfg. Co. Inc., 1150 W. Euclid Ave, Palatine, IL 60067, USA) electronic thermometer with Iron-Constantan thermocouple bead probe. Calibration was checked periodically at 0°C and 100°C.

API 20E strips

Identification of enteric bacteria, based on a series of 20 substrate utilisation and biochemical tests

A.2 - Computer software

@RISK™
Risk analysis and simulation add-in for Microsoft® Excel or Lotus® 1-2-3
Palisade Corporation 31 Decker Road, Newfield, NY USA 14867 (607) 277-8000

BestFit™
Probability distribution fitting for Windows®
Palisade Corporation 31 Decker Road, Newfield, NY USA 14867 (607) 277-8000
Food

SAS PROC NLIN routine
Statistical Analysis System, SAS Institute Incorporated, SAS Circle, Cary, North Carolina, USA. Non-linear regression routine written by Dr G. McPherson, Mathematics Department, University of Tasmania

Ultrafit
Biosoft® 49 Bateman st, Cambridge, CB2 1LR, UK

Microsoft Excel 97
Microsoft Corporation, USA
'VLOOKUP' function – used for stochastic modelling, from Microsoft Excel HELP
Searches for a value in the leftmost column of a table, and then returns a value in the same row from a column you specify in the table. Use VLOOKUP instead of HLOOKUP when your comparison values are located in a column to the left of the data you want to find.
Syntax
VLOOKUP(lookup_value,table_array,col_index_num,range_lookup)
Lookup_value is the value to be found in the first column of the array.
Lookup_value can be a value, a reference, or a text string.
Table_array is the table of information in which data is looked up. Use a reference to a range or a range name, such as Database or List.

If range_lookup is TRUE, the values in the first column of table_array must be placed in ascending order: ..., -2, -1, 0, 1, 2, ..., A-Z, FALSE, TRUE; otherwise VLOOKUP may not give the correct value. If range_lookup is FALSE, table_array does not need to be sorted.

- You can put the values in ascending order by choosing the Sort command from the Data menu and selecting Ascending.
- The values in the first column of table_array can be text, numbers, or logical values.
- Uppercase and lowercase text are equivalent.

Col_index_num is the column number in table_array from which the matching value must be returned. A col_index_num of 1 returns the value in the first column in table_array; a col_index_num of 2 returns the value in the second column in table_array, and so on. If col_index_num is less than 1, VLOOKUP returns the #VALUE! error value; if col_index_num is greater than the number of columns in table_array, VLOOKUP returns the #REF! error value.

Range_lookup is a logical value that specifies whether you want VLOOKUP to find an exact match or an approximate match. If TRUE or omitted, an approximate match is returned. In other words, if an exact match is not found, the next largest value that is less than lookup_value is returned. If FALSE, VLOOKUP will find an exact match. If one is not found, the error value #N/A is returned.

Remarks

- If VLOOKUP can't find lookup_value, and range_lookup is TRUE, it uses the largest value that is less than or equal to lookup_value.
- If lookup_value is smaller than the smallest value in the first column of table_array, VLOOKUP returns the #N/A error value.
- If VLOOKUP can't find lookup_value, and range_lookup is FALSE, VLOOKUP returns the #N/A value.

Micromodel Version 2.0

Food Micromodel Ltd. Leatherhead Food Research Association
Randalls Rd, Leatherhead, Surrey, KT22 7RY, UK

This program is the result of a collaboration coordinated by the Ministry of Agriculture, Fisheries and Food (MAFF) in the United Kingdom. It was originally developed as a consultancy service but is now available as a software package. It contains models for *Aeromonas hydrophila, Bacillus cereus, Bacillus licheniformis, Bacillus subtilus, Staphylococcus aureus, Campylobacter jejuni, Clostridium perfringens,* non-proteolytic *Clostridium botulinum, Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella* and *Yersinia enterocolitica.* The growth models take into account temperature, water activity and pH, as well as the effect of organic acids, carbon dioxide and sodium nitrite. There are also survival and thermal death models available in the software. Predictions can be gathered based on the influence of either single factors or combinations of factors at a range of values, with up to 1000 combinations available at any one time. Results are presented in either a table or can be graphed in both 2 and 3 dimensional forms.

Pathogen Modeling Program Version 5.1 (1998)

Microbial Food Safety Research Unit, USDA ARS Eastern Regional Research Center
600 East Mermaid Lane, Philadelphia, PA, USA, 19118

Up-to-date versions of the software are available free-of-charge from the USDA web site: http://www.arserrc.gov/

This predictive microbiology application program was designed by R.L. Buchanan and R.C. Whiting as a research and instructional tool for estimating the effects of temperature, pH, water activity and sodium nitrite concentration on the growth and survival of foodborne pathogens. There are growth models for the following organisms:

Listeria monocytogenes, Salmonella, Escherichia coli O157:H7, *Staphylococcus aureus, Shigella flexneri, Aeromonas hydrophila, Yersinia enterocolitica* and *Bacillus cereus.*

There is also a "Time to toxigenesis" model for *Clostridium botulinum* and non-thermal inactivation models for *L. monocytogenes, S. aureus* and *Salmonella.*

The conditions of interest are entered into the program and the Gompertz parameters are generated and displayed including growth rate, generation time and lag phase duration, or a graph can be constructed showing growth of the organism (or group of organisms) over a specified time period.

The results gained from the models contained within the Food MicroModel database have been compared against scientific literature and evaluated in actual food products.

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Appendix B – Australian Standard Methods

B1 – Standard methods used for microbial enumeration from cheese samples

AS1766.1.2 (1991) - General procedures and techniques - Preparations of dilutions

AS1766.1.3 (1991) - General procedures and techniques - Colony count - Pour plate method

AS1766.1.4 (1991) - General procedures and techniques - Colony count - Surface spread method

AS1766.2.1 (1991) -Examination for specific organisms - Standard plate count

AS1766.2.1 (1997) -Examination for specific organisms - Yeast and mould

AS1766.2.3 (1992) - Examination for specific organisms - Coliforms and *Escherichia coli*

AS1766.2.4 (1994) - Examination for specific organisms - Coagulase-positive staphylococci

AS1766.2.5 (1991) - Examination for specific organisms - Salmonellae

AS1766.2.15 (Int) (1991) - Examination for specific organisms – *Listeria monocytogenes* in dairy products

AS1766.3.15 (1994) - Examination of specific products - Cheese

B2 – Validation of interim *L. monocytogenes* method

The isolation of *L. monocytogenes* from cheese was the subject of several publications (Doyle & Schoeni, 1987; Lovett, 1988) and there has been conjecture on the best methods for the isolation of injured *L. monocytogenes* cells (Sutherland & Porritt, 1997).

A *L. monocytogenes* inoculum was prepared according to the method outlined in Appendix C, except that the culture was incubated for 44 hr, allowing the initiation of cell death due to stationary phase. The validation was conducted in this way to test the method for detection of injured cells. Standard procedures were then followed to prepare the inoculum, which was then diluted to appropriate levels. A 25 g cheese sample was placed into a stomacher bag, and 0.1 mL of the inoculum added. This was stomached for 4-5 min to achieve homogenous distribution throughout the cheese. 225 mL of LEB was then added, the bag folded over, sealed with tape and placed in the 30°C incubator for 7 days. To enumerate the inoculum of *L. monocytogenes* cells being introduced onto the cheese, 0.1 mL of the inoculum was spread plated onto four LSA plates. An un-inoculated cheese was included as a negative control. Samples were taken from the cheeses after 24 hr, 48 hr and 7 days and streaked onto four LSA plates.

Cheese samples that contained at least an average of one *L. monocytogenes* cell/10 g cheese (average count over four LSA plates of 2.5 colonies per 25 g cheese) gave positive results after only 24 hr enrichment. The lower dilution did not give a positive result, but it was found that when the corresponding LSA plates were examined, two of the four plates did not show any colonies. Therefore, it was possible that no *L. monocytogenes* cells were introduced onto the cheese. The sensitivity of the standard method appears satisfactory for the needs of the current experiment, and tends to give positive results even when very small levels of *L. monocytogenes* are present.

Appendix C – Culture maintenance and preparation of inoculum

C.1 - Culture maintenance

Stock cultures were maintained at -70°C on the surface of plastic embroidery beads held in a 2 mL screw-cap vial, by the method of Jones (1984). The beads were washed in tap water with detergent, followed by dilute HCl to neutralise alkalinity, and then washed several times in tap water, followed by distilled water and dried. Approximately 20 beads were placed in each vial, which was then sterilised at 12°C for 15 min. Cultures were maintained in triplicate, one used for routine recovery, the others held in reserve. A single colony from each bacterial strain was grown overnight on an appropriate non selective agar plate under optimal conditions. Approximately 1 mL of sterile 15% (v/v) glycerol in nutrient broth was dispensed onto the plate. The growth was emulsified with a wire loop to make a thick suspension, and then 0.1 mL aseptically transferred with a pasteur pipette into the prepared vials. The suspension was aspirated several times to ensure the air bubbles inside the bead were displaced. Excess suspension was removed to prevent the beads sticking together when frozen. Recovery was achieved by removing a bead with sterile forceps and rubbing over the surface of a suitable non selective agar medium and also a selective agar (to check the purity and identity), which were then incubated appropriately.

C.2 - Preparation of inoculum

A wild-type *L. monocytogenes* strain (isolated from mussel, obtained from S. Soontranon - University of Tasmania) was used in validation studies. The strain exhibited ß haemolysis on chocolate blood agar, therefore indicating that it was a pathogenic strain (Soontranon *pers comm*). To subculture, a frozen bead was rubbed onto a TSB-YE plate, grown overnight and then placed into 50 mL TSB-YE broth. Cells were grown overnight at 25°C to stationary phase, 1 mL removed and subcultured into another 50 mL TSB-YE to prepare the inoculum. This was grown overnight for 20 hours and 10 mL placed into a centrifuge tube and spun for 15 min at 6000 rpm (head size 15 cm). The supernatant was poured off, and the resulting pellet resuspended in 0.1% peptone water, and the centrifugation repeated. This procedure was repeated twice more. The initial culture was assumed to be in stationary phase, and therefore contain around 10^9 cfu/mL. The inoculum was serially diluted based on this assumption to a level in the order of 10^6 cfu/mL.

Appendix D – Calculations: Water activity, Generation time, Lag phase duration and Risk assessment model

Table D.1 – Calculation of salt concentration from measured a_w

(adapted from Resnik & Chirife, 1988)

Total % NaCl	Calculated water activity	Total % NaCl	Calculated water activity
0.0	1.000	10.5	0.933
0.5	0.997	11.0	0.930
1.0	0.994	11.5	0.926
1.5	0.991	12.0	0.923
2.0	0.989	12.5	0.919
2.5	0.986	13.0	0.916
3.0	0.983	13.5	0.913
3.5	0.980	14.0	0.909
4.0	0.977	14.5	0.906
4.5	0.974	15.0	0.902
5.0	0.970	15.5	0.899
5.5	0.967	16.0	0.896
6.0	0.964	16.5	0.892
6.5	0.960	17.0	0.889
7.0	0.957	17.5	0.885
7.5	0.954	18.0	0.882
8.0	0.950	18.5	0.878
8.5	0.947	19.0	0.875
9.0	0.943	19.5	0.872
9.5	0.940	20.0	0.868
10.0	0.937	20.5	0.865

D.2 - Calculation of Generation time and lag phase duration

From McMeekin *et al.* (1993) pg 80-86, where these expressions are derived from first principles. The Gompertz equation used for modelling bacterial growth is of the form shown in Eqn A.1. The exponential growth rate may be determined by calculating the slope of the tangent to the modified Gompertz curve, at time *M*, which is the time at which the growth rate is the fastest (steepest part of the curve).

$$\log N_{(t)} = A + D\exp\{-\exp[-B(t-M)]\} \qquad \text{A.1}$$

where:

t = time

$N_{(t)}$ = population density at time (t)

Gompertz parameters are defined differently, depending on whether plate count data / %transmittance data is used;

Table D.2 - Definitions of Gompertz parameters for different data types

	Plate count data	%transmittance data
A	value of the lower asymptote	lower limit of detection of the spectrophotometer or %transmittance of the initial microbial load
B	related to the slope of the curve at *M* such that BD/e is the slope of the tangent	maximum rate of change of %transmittance
M	time at which the exponential growth is maximal	time at which rate of change of %transmittance is maximal
D	difference in value of the upper and lower asymptote	difference between the lower and upper limits of sensitivity of the spectrophotometer

The expressions to calculate generation time are as follows, with Eqn A.2 used for plate count data and Eqn A.3 for %transmittance data.

$$\text{Generation time (plate count data)} = \frac{0.8183}{BD} \qquad \text{A.2}$$

$$\text{Generation time (\%T data)} = \frac{66.59}{BD} \qquad \text{A.3}$$

In all calculations of generation time, a calibration factor of 1.13 was introduced to account for the apparent under-prediction associated with the use of the modified Gompertz function (Section 1.4.2.1). Eqn A.4 was used for the calculation of lag times.

$$\text{Lag phase duration} = M - \frac{1}{B}\left[1 - \exp\{1 - \exp(BM)\}\right] \qquad \text{A.4}$$

D3 – Detailed Risk Assessment Model structure

The model used in the detailed risk assessment is shown in Figs D1-D4. These screen captures present the structure of the model, and details of the input parameters and output values will be provided here. Model equations are shown in the first two lines, to demonstrate how calculations are conducted.

Fig D1 shows the inputs of the cheesemaking parameters into the risk assessment model. At each 3 minute interval, the average temperature, pH and salt concentration are presented to the model, plus the standard deviation multiplied by the value supplied by the Normal (0,1) distribution in the @RISK software. This results in the calculation of the Model temperature, pH and %NaCl (Columns D, H, L) which are used for the calculation of *L. monocytogenes* growth parameters (Fig D2). A value is chosen from within the Normal distribution for each of the 10,000 iterations used for each simulation. The same value from the Normal distribution is then used for the entire iteration.

Fig D2 presents the calculation of *L. monocytogenes* generation time and the resultant level of *L. monocytogenes* in the cheese. The calculated Model process parameters are entered into the Murphy model (Columns Q, R, S, T), to calculate the natural logarithmic derivation of Gompertz parameters B, C, and M (as defined in Eqns 3.2 – 3.4 in Section 3.2). The Generation time is then calculated from these values as shown in Column U. The Step (GT) (Column N) is the number of generations of *L. monocytogenes* growth per unit time (ie 3 minutes). These values are summed to calculate the level of *L. monocytogenes* present in the cheese (Column P), plus the starting level of contamination (cell V2 in Fig D3).

Fig D3 presents the input parameter distributions and derived estimates, as described in Tables 2.1-2.3 from Section 2.4.1. The model was constructed so that the predicted level of *L. monocytogenes* was calculated at any given time (as shown in Fig D2). This value was calculated without regard for time of contamination, and was used for the simple risk assessment model. For the detailed risk assessment outcomes, the 'Level before contamination' utilised the Excel 'LOOKUP' function to locate the time (at random) at which contamination was predicted to occur (uniform distribution, cell V6). This level of *L. monocytogenes* (cell V9), and the equivalent growth spent in lag phase (cell V12) was then subtracted from cell V15 (the end of production and storage stage) to calculate the 'Level at end of storage' (cell V18).

The Excel 'LOOKUP' function (cell X2) was also used to calculate the predicted 'Level at time of consumption' (cell W6), in a similar fashion. However, for this parameter the specified distribution was a Triangular (cell W2). The proviso was put on the calculated value in both cell V18 and W6 that the final predicted level of *L. monocytogenes* could not be less than the initial starting value (V2), and not more than 8 (i.e. 10^8 cfu/g). Also shown in Fig D3 are the parameter input distributions for 'Serving size' (cell W9) and 'Frequency of contamination' (cell W25). These distributions are used in the final calculation of listeriosis risk in Fig D4. The derived estimates for 'Dose' (cell W12) and 'Number of serves' (cells W22 and X22) are also calculated in this portion of the model, the outputs of which were presented in each Case Study (chapters 4, 5, 6). The 'Number of serves per year' is calculated from the annual production, divided by the 'Serving size'. The probability of infection, using R-values as defined by Ross (*unpublished*) (cell W15) and Buchanan *et al.* (1997a) (cell W16), are also shown in Fig D3. The probability is determined by the Dose-response equation (as defined in Eqn 2.2), subtracting from one, the exponential of multiplying the R-value by the Dose.

Fig D4 shows the model calculations to predict the number of listeriosis cases as a result of the consumption of the cheese brand under consideration. The susceptible groups are listed in Column Y, and the proportion of the population they constitute in Column Z. The number of people (Column AA) is calculated by multiplying Column Z by the population of Tasmania (Cell AA23) and rest of Australia (not shown). The proportion who consume cheese is listed in Column AB (from the survey results of McLennan & Podger (1999)). Therefore, the number of consumers of the cheese brand is then calculated by multiplying Column AA by Column AB, and made brand specific by also including a factor for Market share (Column AC). The relative susceptibilities (from Chapter 2) are listed in Column AD, and the calculated annual cheese consumption listed in Column AE (calculated by averaging the age groupings from Table 2.6). The number of listeriosis case per annum are then calculated for each population grouping, according to the equation shown in Column AF.

D4 – Predictive Model Evaluation – Literature data

All literature data points used in the evaluation of *L. monocytogenes* predictive growth models (Chapter 3) are listed in Table D3.

Figure D1 – Detailed risk assessment model – process parameter modelling

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U21 = =((LOG10(2)*T21)/((T21^Q21)*(T21^R21)))

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	**Brie - complicated risk assessment, Production**												
2	**Time**	**Temp**	**Std**	**Model**	**Temperature**	**pH**	**Std**	**Model pH**	**pH**	**%NaCl**	**Std**	**Model**	**%NaCl**
3			**Dev**	**temp**	**distribution**		**Dev**		**distribution**		**Dev**	**%NaCl**	**distribution**
4	0.00	39.2	0.4	B4+($E$4*C4)	RiskNormal(0,1)	6.45	0.11	F4+($I$4*G4)	RiskNormal(0, 1)	0.79	0.16	J4+($M$4*K4)	RiskNormal(0, 1)
5	0.05	39.1	0.4	B5+($E$4*C5)		6.45	0.10	F5+($I$4*G5)		0.79	0.16	J5+($M$4*K5)	
6	0.10	39.1	0.4	39.1		6.44	0.09	6.44		0.79	0.15	0.79	
7	0.15	39.1	0.4	39.1		6.43	0.08	6.43		0.80	0.15	0.80	
8	0.20	39.1	0.4	39.1		6.43	0.07	6.43		0.80	0.15	0.80	
9	0.25	39.0	0.5	39.0		6.42	0.06	6.42		0.81	0.15	0.81	
10	0.30	39.0	0.5	39.0		6.42	0.05	6.42		0.82	0.15	0.82	
11	0.35	38.9	0.6	38.9		6.42	0.05	6.42		0.83	0.15	0.83	
12	0.40	38.9	0.6	38.9		6.41	0.04	6.41		0.84	0.16	0.84	
13	0.45	38.7	0.8	38.7		6.41	0.04	6.41		0.85	0.17	0.85	
14	0.50	38.7	0.8	38.7		6.40	0.05	6.40		0.86	0.17	0.86	
15	0.55	38.7	0.8	38.7		6.40	0.05	6.40		0.87	0.19	0.87	
16	0.60	38.7	0.8	38.7		6.39	0.06	6.39		0.89	0.20	0.89	
17	0.65	38.5	1.2	38.5		6.38	0.07	6.38		0.90	0.21	0.90	
18	0.70	38.6	1.1	38.6		6.38	0.08	6.38		0.91	0.22	0.91	
19	0.75	38.5	1.2	38.5		6.37	0.09	6.37		0.92	0.23	0.92	
20	0.80	38.5	1.3	38.5		6.36	0.10	6.36		0.93	0.25	0.93	
21	0.85	38.2	1.4	38.2		6.36	0.11	6.36		0.95	0.26	0.95	
22	0.90	38.3	1.3	38.3		6.36	0.09	6.36		0.96	0.27	0.96	
23	0.95	38.2	1.3	38.2		6.37	0.07	6.37		0.97	0.29	0.97	
24	1.00	38.1	1.4	38.1		6.38	0.06	6.38		0.97	0.28	0.97	
25	1.05	38.1	1.3	38.1		6.38	0.05	6.38		0.98	0.26	0.98	

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Figure D2 – Detailed risk assessment – calculation of generation time and *L. monocytogenes* level

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Q4 =-48.0193+0.5612*D4+0.1934*L4+18.0587*H4-0.0098*D4^2-0.0375*L4^2-2.6085*H4^2-0.0214*D4*L4-0.0442*D4*H4+0.1272*H4^3+0.003*D4*H4*L4+0.0008*D4^2*H4

	N	O						
1			Level		Murphy-model parameters			
2	step (GT)	Time	(log cfu/g)	ln B	ln C	ln M	e=	GT=
3								
4	0.000	0.00	V2	#VALUE!	1.390	1.155	2.72	((LOG10(2)*T4)/((T4^Q4)*(T4^R4)))
5	(((O5-O4)/U5))	0.05	((LOG(2^(SUM($N$4:N5)))+$P$4))	-2.156	1.390	1.155	2.72	((LOG10(2)*T5)/((T5^Q5)*(T5^R5)))
6	(((A6-A5)/U6))	0.10	((LOG(2^(SUM($N$4:N6)))+$P$4))	-2.156	1.390	1.155	2.72	((LOG10(2)*T6)/((T6^Q6)*(T6^R6)))
7	0.028	0.15	-0.9914	-2.155	1.391	1.156	2.72	1.8
8	0.029	0.20	-0.9829	-2.154	1.391	1.157	2.72	1.8
9	0.029	0.25	-0.9828	-2.147	1.392	1.158	2.72	1.7
10	0.029	0.30	-0.9827	-2.146	1.392	1.160	2.72	1.7
11	0.029	0.35	-0.9826	-2.139	1.392	1.161	2.72	1.7
12	0.029	0.40	-0.9826	-2.138	1.392	1.163	2.72	1.7
13	0.030	0.45	-0.9824	-2.120	1.393	1.164	2.72	1.7
14	0.030	0.50	-0.9822	-2.119	1.393	1.166	2.72	1.7
15	0.030	0.55	-0.9822	-2.118	1.393	1.168	2.72	1.7
16	0.030	0.60	-0.9822	-2.117	1.394	1.170	2.72	1.7
17	0.030	0.65	-0.9820	-2.098	1.395	1.172	2.72	1.7
18	0.030	0.70	-0.9818	-2.103	1.395	1.174	2.72	1.7
19	0.030	0.75	-0.9818	-2.096	1.395	1.176	2.72	1.6
20	0.031	0.80	-0.9817	-2.089	1.396	1.178	2.72	1.6
21	0.031	0.85	-0.9814	-2.065	1.397	1.181	2.72	1.6
22	0.031	0.90	-0.9812	-2.071	1.395	1.181	2.72	1.6
23	0.031	0.95	-0.9812	-2.065	1.395	1.183	2.72	1.6
24	0.032	1.00	-0.9811	-2.055	1.395	1.182	2.72	1.6
25	0.031	1.05	-0.9810	-2.060	1.394	1.184	2.72	1.6

Sheet1 Sheet2 Sheet3

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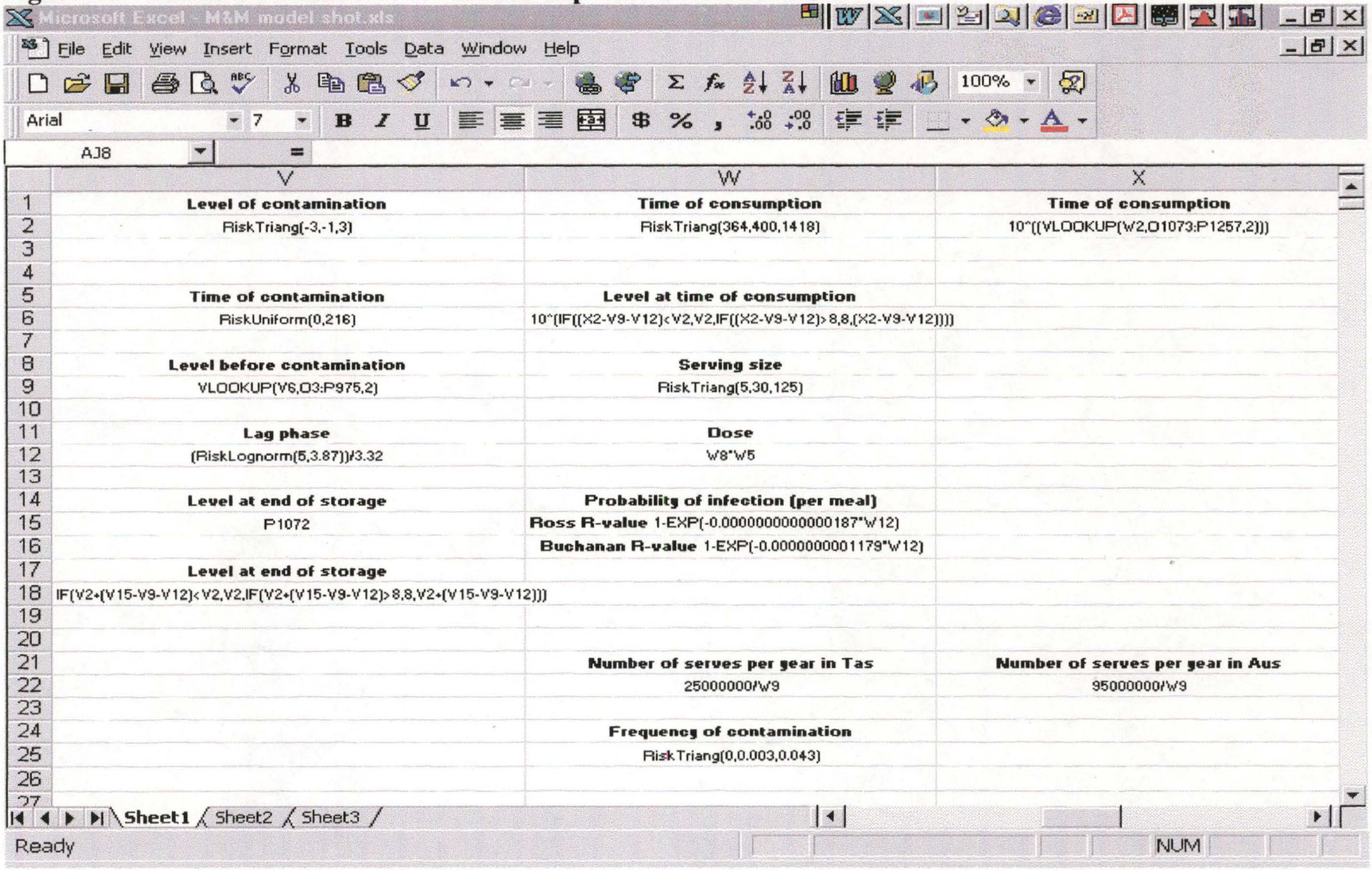
Figure D3 – Detailed risk assessment model - input distribution screen



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AJ8 =

	V	W	X
1	**Level of contamination**	**Time of consumption**	**Time of consumption**
2	RiskTriang(-3,-1,3)	RiskTriang(364,400,1418)	10^((VLOOKUP(W2,O1073:P1257,2)))
3			
4			
5	**Time of contamination**	**Level at time of consumption**	
6	RiskUniform(0,216)	10^(IF((X2-V9-V12)<V2,V2,IF((X2-V9-V12)>8,8,(X2-V9-V12))))	
7			
8	**Level before contamination**	**Serving size**	
9	VLOOKUP(V6,O3:P975,2)	RiskTriang(5,30,125)	
10			
11	**Lag phase**	**Dose**	
12	(RiskLognorm(5,3.87))/3.32	W8*W5	
13			
14	**Level at end of storage**	**Probability of infection (per meal)**	
15	P1072	**Ross R-value** 1-EXP(-0.00000000000000187*W12)	
16		**Buchanan R-value** 1-EXP(-0.0000000001179*W12)	
17	**Level at end of storage**		
18	IF(V2+(V15-V9-V12)<V2,V2,IF(V2+(V15-V9-V12)>8,8,V2+(V15-V9-V12)))		
19			
20			
21		**Number of serves per year in Tas**	**Number of serves per year in Aus**
22		25000000/W9	95000000/W9
23			
24		**Frequency of contamination**	
25		RiskTriang(0,0.003,0.043)	
26			
27			

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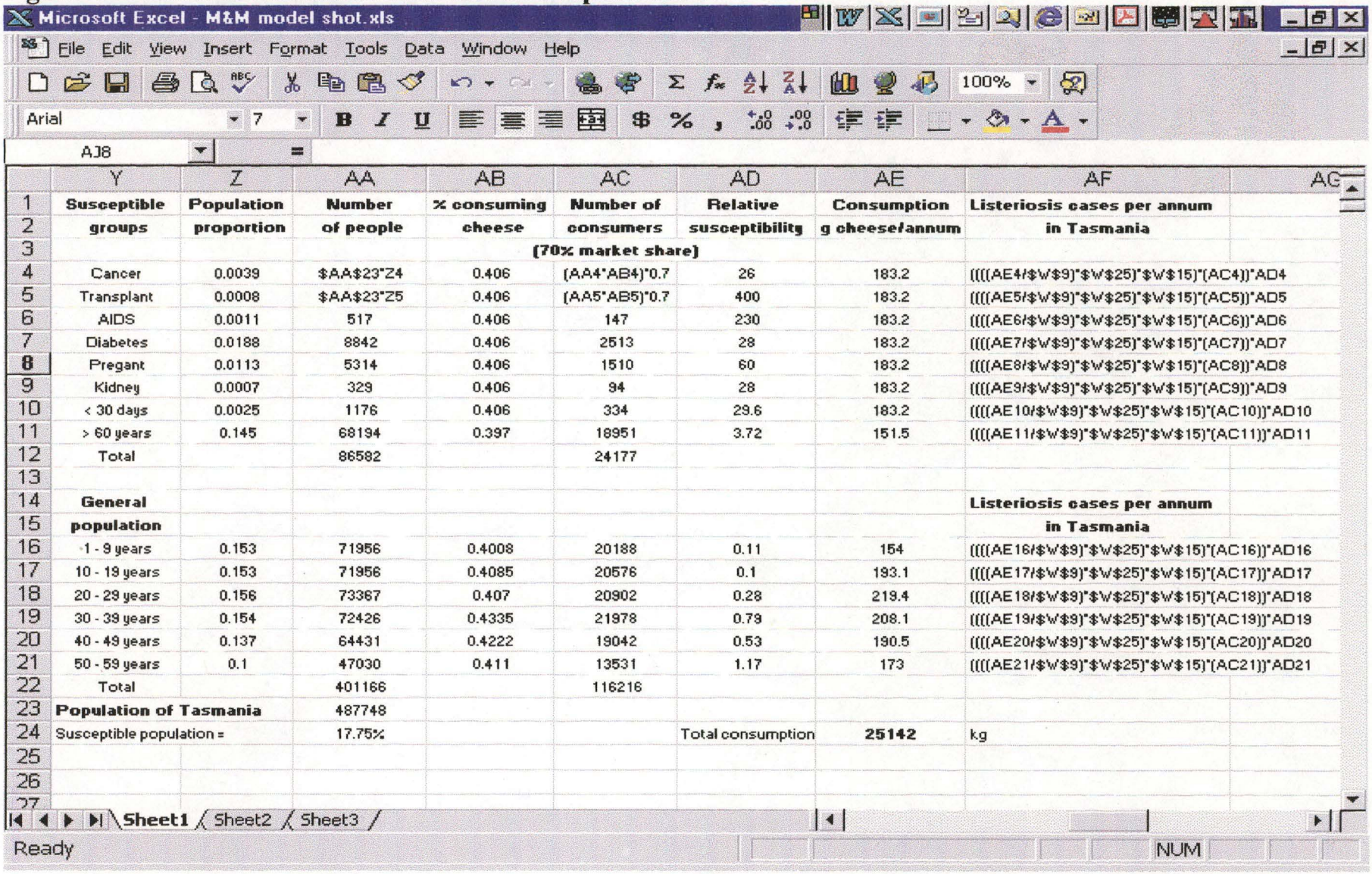
Figure D4 - Detailed risk assessment model - output screen



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AJ8 =

	Y	Z	AA	AB	AC	AD	AE	AF	AG
1	**Susceptible**	**Population**	**Number**	**% consuming**	**Number of**	**Relative**	**Consumption**	**Listeriosis cases per annum**	
2	**groups**	**proportion**	**of people**	**cheese**	**consumers**	**susceptibility**	**g cheese/annum**	**in Tasmania**	
3				**(70% market share)**					
4	Cancer	0.0039	$AA$23*Z4	0.406	(AA4*AB4)*0.7	26	183.2	((((AE4/$W$9)*$W$25)*$W$15)*(AC4))*AD4	
5	Transplant	0.0008	$AA$23*Z5	0.406	(AA5*AB5)*0.7	400	183.2	((((AE5/$W$9)*$W$25)*$W$15)*(AC5))*AD5	
6	AIDS	0.0011	517	0.406	147	230	183.2	((((AE6/$W$9)*$W$25)*$W$15)*(AC6))*AD6	
7	Diabetes	0.0188	8842	0.406	2513	28	183.2	((((AE7/$W$9)*$W$25)*$W$15)*(AC7))*AD7	
8	Pregant	0.0113	5314	0.406	1510	60	183.2	((((AE8/$W$9)*$W$25)*$W$15)*(AC8))*AD8	
9	Kidney	0.0007	329	0.406	94	28	183.2	((((AE9/$W$9)*$W$25)*$W$15)*(AC9))*AD9	
10	< 30 days	0.0025	1176	0.406	334	29.6	183.2	((((AE10/$W$9)*$W$25)*$W$15)*(AC10))*AD10	
11	> 60 years	0.145	68194	0.397	18951	3.72	151.5	((((AE11/$W$9)*$W$25)*$W$15)*(AC11))*AD11	
12	Total		86582		24177				
13									
14	**General**							**Listeriosis cases per annum**	
15	**population**							**in Tasmania**	
16	1 - 9 years	0.153	71956	0.4008	20188	0.11	154	((((AE16/$W$9)*$W$25)*$W$15)*(AC16))*AD16	
17	10 - 19 years	0.153	71956	0.4085	20576	0.1	193.1	((((AE17/$W$9)*$W$25)*$W$15)*(AC17))*AD17	
18	20 - 29 years	0.156	73367	0.407	20902	0.28	219.4	((((AE18/$W$9)*$W$25)*$W$15)*(AC18))*AD18	
19	30 - 39 years	0.154	72426	0.4335	21978	0.79	208.1	((((AE19/$W$9)*$W$25)*$W$15)*(AC19))*AD19	
20	40 - 49 years	0.137	64431	0.4222	19042	0.53	190.5	((((AE20/$W$9)*$W$25)*$W$15)*(AC20))*AD20	
21	50 - 59 years	0.1	47030	0.411	13531	1.17	173	((((AE21/$W$9)*$W$25)*$W$15)*(AC21))*AD21	
22	Total		401166		116216				
23	**Population of Tasmania**		487748						
24	Susceptible population =		17.75%			Total consumption	**25142**	kg	
25									
26									
27									

Sheet1 Sheet2 Sheet3

Ready NUM

Table D3 - Predictive Model evaluation - Literature data													
Ref	Strain	Food	Temp	pH	%NaCl	aw	Atm	Assumptions	observed GT	Ross	Murphy	PMP	FMM
1	F5069	Whole milk	35	6.4	0.8	0.995	aerobic		0.43	0.43	1.28	0.30	0.46
1	F5069	Whole milk	21	6.4	0.8	0.995	aerobic		1.18	1.18	1.30	0.90	0.88
1	F5069	Whole milk	10	6.4	0.8	0.995	aerobic		3.71	5.06	5.52	4.00	4.71
1	F5069	Whole milk	4	6.4	0.8	0.995	aerobic		12.10	29.37	22.71	12.10	18.04
2		Raw milk	4	6.4	0.8	0.995		aw, pH	25.30	29.37	22.71	12.10	18.04
2		Raw milk	10	6.4	0.8	0.995		aw, pH	10.80	5.06	5.52	4.00	4.71
2		Raw milk	15	6.4	0.8	0.995		aw, pH	7.40	2.29	2.41	1.90	1.94
3	DA 3	Milk	5	6.5	0.8	0.995	aerobic		18.07	19.26	17.23	9.70	13.76
3	Brie 1	Milk	5	6.5	0.8	0.995	aerobic		13.46	19.26	17.23	9.70	13.76
3	3x clinical/food	Milk	22	6.5	0.8	0.995	aerobic		1.33	1.08	1.26	0.80	0.78
3	3x clinical/food	Milk	22	6.5	0.8	0.995	aerobic		1.33	1.08	1.26	0.80	0.78
4	Scott A	Whole Milk	10	6.4	0.8	0.995	aerobic	aw, pH	10.10	5.06	5.52	4.00	4.71
4	Scott A	Whole Milk	10	6.4	0.8	0.995	aerobic	aw, pH	8.05	5.06	5.52	4.00	4.71
4	Scott A	Whole Milk	10	6.4	0.8	0.995	aerobic	aw, pH	7.13	5.06	5.52	4.00	4.71
4	Scott A	Skim Milk	10	6.4	0.8	0.995	aerobic	aw, pH	10.00	5.06	5.52	4.00	4.71
4	Scott A	Skim Milk	10	6.4	0.8	0.995	aerobic	aw, pH	7.95	5.06	5.52	4.00	4.71
4	Scott A	Skim Milk	10	6.4	0.8	0.995	aerobic	aw, pH	7.02	5.06	5.52	4.00	4.71
4	Scott A	Nonfat Milk Solids	10	6.4	0.8	0.995	aerobic	aw, pH	10.00	5.06	5.52	4.00	4.71
4	Scott A	Nonfat Milk Solids	10	6.4	0.8	0.995	aerobic	aw, pH	8.67	5.06	5.52	4.00	4.71
4	Scott A	Nonfat Milk Solids	10	6.4	0.8	0.995	aerobic	aw, pH	8.46	5.06	5.52	4.00	4.71
4	Scott A	Whole milk	10	6.4	0.8	0.995	aerobic	aw, pH	6.60	5.06	5.52	4.00	4.71
5	NCTC 5348	Pasteurised milk	3	6.85	0.17	0.996	aerobic	aw	30.00	49.35	30.78		21.71
5	NCTC 5348	Pasteurised milk	6	6.85	0.17	0.996	aerobic	aw	26.53	13.38	14.44	7.60	10.41
5	NCTC 5348	Pasteurised milk	9	6.85	0.17	0.996	aerobic	aw	12.01	6.12	7.58	4.40	5.38
5	NCTC 5348	UHT milk	3	6.75	0.19	0.996	aerobic	aw	32.06	49.43	30.86		21.71
5	NCTC 5348	UHT milk	6	6.75	0.19	0.996	aerobic	aw	18.02	13.40	14.31	7.6	10.41
5	NCTC 5348	UHT milk	9	6.75	0.19	0.996	aerobic	aw	8.23	6.13	7.44	4.40	5.38
5	NCTC 5348	Cream	3	6.4	0.2	0.996	aerobic	aw	35.78	49.46	31.92		23.44
5	NCTC 5348	Cream	6	6.4	0.2	0.996	aerobic	aw	14.28	13.41	14.20	8.10	11.24

Table D3 - Predictive Model evaluation - Literature data													
Ref	Strain	Food	Temp	pH	%NaCl	aw	Atm	Assumptions	observed GT	Ross	Murphy	PMP	FMM
5	NCTC 5348	Cream	10	6.4	0.2	0.996	aerobic	aw	2.72	4.99	5.80	4.00	4.71
5	NCTC 5348	Butter	3	7	1.39	0.990	aerobic	aw	49.60	54.44	27.37		21.11
5	NCTC 5348	Butter	6	7	1.39	0.990	aerobic	aw	26.40	14.76	13.09	8.10	10.18
5	NCTC 5348	Camembert	4	6.4	1.8	0.987	aerobic	aw	43.23	33.07	21.49	13.50	17.84
5	NCTC 5348	Camembert	10	6.4	1.8	0.987	aerobic	aw	3.59	5.70	5.30	4.50	4.73
6		Heat-treated whe	7	6.5	0.8	0.995		aw	12.00	10.11	10.55	6.60	8.65
6		Heat-treated whe	12	6.5	0.8	0.995		aw	6.00	3.55	3.87	2.80	3.14
6		Heat-treated whe	20	6.5	0.8	0.995		aw	4.00	1.30	1.44	0.90	0.96
6		Heat-treated whe	30	6.5	0.8	0.995		aw	0.67	0.58	1.08	0.40	0.47
7	Scott A	6% salted whey	4	5.6	6.2	0.962	aerobic		46.81	394.01	59.00	42.70	37.53
7	Scott A	skim milk	4	6.1	0.8	0.995	aerobic		45.23	34.26	24.44	14.00	20.07
7	CA	6% salted whey	4	5.6	6.2	0.962	aerobic		37.49	394.01	59.00	42.70	37.53
7	CA	skim milk	4	6.1	0.8	0.995	aerobic		49.43	34.26	24.44	14.00	20.07
7	Scott A	6% salted whey	22	5.6	6.2	0.962	aerobic		3.67	14.43	3.50	2.50	1.99
7	Scott A	skim milk	22	6.1	0.8	0.995	aerobic		4.31	1.25	1.15	0.80	0.89
7	CA	6% salted whey	22	5.6	6.2	0.962	aerobic		3.56	14.43	3.50	2.50	1.99
7	CA	skim milk	22	6.1	0.8	0.995	aerobic		4.42	1.25	1.15	0.80	0.89
8	Scott A	Myzithra	5	6.50	0	0.998	aerobic		16.16	18.46	18.64	9.30	14.16
8	Scott A	Anthotyros	5	6.41	1.42	0.992	aerobic		20.16	20.14	16.67	10.20	13.95
8	Scott A	Manouri	5	6.31	2.28	0.987	aerobic		17.81	21.79	16.59	11.40	14.41
8	Scott A	Myzithra	12	6.50	0	0.998	aerobic		5.65	3.40	4.15	2.70	3.21
8	Scott A	Anthotyros	12	6.41	1.42	0.992	aerobic		5.17	3.71	3.71	3.00	3.20
8	Scott A	Manouri	12	6.31	2.28	0.987	aerobic		5.68	4.01	3.67	3.30	3.35
8	Scott A	Myzithra	22	6.50	0	0.998	aerobic		1.93	1.03	1.33	0.70	0.78
8	Scott A	Anthotyros	22	6.41	1.42	0.992	aerobic		1.95	1.12	1.20	0.80	0.80
8	Scott A	Manouri	22	6.31	2.28	0.987	aerobic		1.79	1.22	1.20	0.90	0.85
8	California	Myzithra	5	6.50	0	0.998	aerobic		16.89	18.46	18.64	9.50	14.16
8	California	Anthotyros	5	6.41	1.42	0.992	aerobic		18.19	20.14	16.67	10.40	13.95
8	California	Manouri	5	6.31	2.28	0.987	aerobic		18.48	21.79	16.59	11.70	14.41

Table D3 - Predictive Model evaluation - Literature data													
Ref	Strain	Food	Temp	pH	%NaCl	aw	Atm	Assumptions	observed GT	Ross	Murphy	PMP	FMM
8	California	Myzithra	12	6.50	0	0.998	aerobic		5.35	3.40	4.15	2.80	3.21
8	California	Anthotyros	12	6.41	1.42	0.992	aerobic		5.07	3.71	3.71	3.00	3.20
8	California	Manouri	12	6.31	2.28	0.987	aerobic		5.81	4.01	3.67	3.40	3.35
8	California	Myzithra	22	6.50	0	0.998	aerobic		2.70	1.03	1.33	0.70	0.78
8	California	Anthotyros	22	6.41	1.42	0.992	aerobic		2.55	1.12	1.20	0.80	0.80
8	California	Manouri	22	6.31	2.28	0.987	aerobic		1.68	1.22	1.20	0.90	0.85
9	V7	Skim milk	13	6.5	1.5	0.991	aerobic	aw	5.05	3.22	3.18	2.60	2.62
9	V7	Skim milk	13	5.6	0.8	0.995	aerobic		5.17	21.22	3.88	4.10	3.94
9	V7	Skim milk	30	6.5	0.8	0.995	aerobic		0.87	0.58	1.08	0.40	0.47
9	V7	Skim milk	35	5.6	0.8	0.995	aerobic		0.80	3.00	1.36	0.60	0.67
10	Scott A	UHT milk	12	6.6	0	0.997		aw, pH	4.31	3.45	4.24	2.70	3.11
10	Scott A	UHT milk	12	6.6	0	0.997		aw, pH	3.63	3.45	4.24	2.70	3.11
10	Scott A	UHT milk	12	6.6	0	0.997		aw, pH	4.79	3.45	4.24	2.70	3.11
10	Scott A	UHT milk	12	6.6	0.5	0.994		aw, pH	4.06	3.58	4.04	2.80	3.06
10	Scott A	UHT milk	12	6.6	0.5	0.994		aw, pH	3.98	3.58	4.04	2.80	3.06
10	Scott A	UHT milk	12	6.6	0.5	0.994		aw, pH	3.93	3.58	4.04	2.80	3.06
10	Scott A	UHT milk	12	6.6	4.5	0.971		aw, pH	6.55	5.42	4.56	4.20	3.98
10	Scott A	UHT milk	12	6.6	4.5	0.971		aw, pH	6.73	5.42	4.56	4.20	3.98
10	Scott A	UHT milk	12	6.6	4.5	0.971		aw, pH	7.76	5.42	4.56	4.20	3.98
10	Scott A	UHT milk	19	6.6	0	0.997		aw, pH	1.68	1.40	1.73	1.00	1.07
10	Scott A	UHT milk	19	6.6	0	0.997		aw, pH	1.62	1.40	1.73	1.00	1.07
10	Scott A	UHT milk	19	6.6	0	0.997		aw, pH	1.65	1.40	1.73	1.00	1.07
10	Scott A	UHT milk	19	6.6	0.5	0.994		aw, pH	1.61	1.45	1.66	1.10	1.06
10	Scott A	UHT milk	19	6.6	0.5	0.994		aw, pH	1.67	1.45	1.66	1.10	1.06
10	Scott A	UHT milk	19	6.6	0.5	0.994		aw, pH	1.85	1.45	1.66	1.10	1.06
10	Scott A	UHT milk	19	6.6	4.5	0.971		aw, pH	2.03	2.20	1.95	1.60	1.45
10	Scott A	UHT milk	19	6.6	4.5	0 971		aw, pH	1.98	2.20	1.95	1.60	1.45
10	Scott A	UHT milk	19	6.6	4.5	0.971		aw, pH	2.08	2.20	1.95	1.60	1.45
10	Scott A	UHT milk	28	6.6	0	0.997		aw, pH	1.00	0.65	1.16	0.40	0.49

Table D3 - Predictive Model evaluation - Literature data													
Ref	Strain	Food	Temp	pH	%NaCl	aw	Atm	Assumptions	observed GT	Ross	Murphy	PMP	FMM
10	Scott A	UHT milk	28	6.6	0	0.997		aw, pH	1.05	0.65	1.16	0.40	0.49
10	Scott A	UHT milk	28	6.6	0	0.997		aw, pH	1.10	0.65	1.16	0.40	0.49
10	Scott A	UHT milk	28	6.6	0.5	0.994		aw, pH	0.93	0.67	1.12	0.40	0.49
10	Scott A	UHT milk	28	6.6	0.5	0.994		aw, pH	1.03	0.67	1.12	0.40	0.49
10	Scott A	UHT milk	28	6.6	0.5	0.994		aw, pH	1.15	0.67	1.12	0.40	0.49
10	Scott A	UHT milk	28	6.6	4.5	0.971		aw, pH	2.50	1.02	1.40	0.70	0.72
10	Scott A	UHT milk	28	6.6	4.5	0.971		aw, pH	2.50	1.02	1.40	0.70	0.72
10	Scott A	UHT milk	28	6.6	4.5	0.971		aw, pH	1.80	1.02	1.40	0.70	0.72
10	Scott A	UHT milk	37	6.6	0	0.997		aw, pH	0.94	0.37		0.30	
10	Scott A	UHT milk	37	6.6	0	0.997		aw, pH	0.63	0.37		0.30	
10	Scott A	UHT milk	37	6.6	0	0.997		aw, pH	0.72	0.37		0.30	
10	Scott A	UHT milk	37	6.6	0.5	0.994		aw, pH	0.68	0.39		0.30	
10	Scott A	UHT milk	37	6.6	0.5	0 994		aw, pH	0.41	0.39		0.30	
10	Scott A	UHT milk	37	6.6	0.5	0.994		aw, pH	0.89	0.39		0.30	
10	Scott A	UHT milk	37	6.6	4.5	0.971		aw, pH	1.33	0.59		0.40	
10	Scott A	UHT milk	37	6.6	4.5	0.971		aw, pH	1.74	0.59		0.40	
10	Scott A	UHT milk	37	6.6	4.5	0.971		aw, pH	1.47	0.59		0.40	
11	Scott A	Whole milk	4	6.5	0.8	0.995	aerobic		30.50	29.37	22.45	11.50	17.58
11	Scott A	Whole milk	8	6.5	0.8	0.995	aerobic		13.00	7.81	8.42	5.40	6.95
11	Scott A	Whole milk	13	6.5	0.8	0.995	aerobic		5.90	3.03	3.29	2.40	2.63
11	Scott A	Chocolate milk	4	6.5	0.8	0.995	aerobic		30.00	29.37	22.45	11.50	17.58
11	Scott A	Chocolate milk	8	6.5	0.8	0.995	aerobic		10.80	7.81	8.42	5.40	6.95
11	V7	Chocolate milk	13	6.4	2.5	0.986	aerobic		4.50	3.49	3.15	2.80	2.76
11	V7	Chocolate milk	8	6.4	2.5	0.986	aerobic		8.60	8.98	8.03	6.40	7.17
11	V7	Chocolate milk	4	6.4	2.5	0.986	aerobic		41.50	33.77	21.38	13.50	17.93
11	V37CE	Chocolate milk	4	6.4	2.5	0.986	aerob c		29.50	33.77	21.38	13.50	17.93
11	V7	Chocolate milk	21	6.4	2.5	0.986	aerobic		1.60	1.36	1.31	1.00	0.92
11	V7	Chocolate milk	35	6.4	2.5	0.986	aerobic		0.68	0.49	1.35	0.30	0.50
11	CA	Whole milk	4	6.4	0.8	0.995	aerobic		30.00	29.37	22.71	11.80	18.04

Table D3 - Predictive Model evaluation - Literature data													
Ref	Strain	Food	Temp	pH	%NaCl	aw	Atm	Assumptions	observed GT	Ross	Murphy	PMP	FMM
11	V7	Whole milk	4	6.4	0.8	0.995	aerobic		36.50	29.37	22.71	11.80	18.04
11	V7	Whole milk	8	6.4	0.8	0.995	aerobic		10.80	7.81	8.40	5.50	7.13
11	V7	Whole milk	13	6.4	0.8	0.995	aerobic		5.00	3.03	3.23	2.40	2.70
11	V7	Whole milk	21	6.4	0.8	0.995	aerobic		1.90	1.18	1.30	0.80	0.88
11	V7	Whole milk	37	6.4	0.8	0.995	aerobic		0.65	0.38		0.30	
11	Scott A	Skim milk	4	6.5	0.8	0.995	aerobic		32.30	29.37	22.45	11.50	17.58
11	Scott A	Skim milk	8	6.5	0.8	0.995	aerobic		12.60	7.81	8.42	5.40	6.95
11	Scott A	Skim milk	13	6.5	0.8	0.995	aerobic		6.13	3.03	3.29	2.40	2.63
11	Scott A	Cream	4	6.5	0.8	0.995	aerobic		32.00	29.37	22.45	11.50	17.58
11	Scott A	Cream	8	6.5	0.8	0.995	aerobic		12.25	7.81	8.42	5.40	6.95
11	Scott A	Cream	13	6.5	0.8	0.995	aerobic		5.83	3.03	3.29	2.40	2.63
11	V7	Cream	4	6.5	0.8	0.995	aerobic		46.00	29.37	22.45	11.50	17.58
11	V7	Cream	8	6.5	0.8	0.995	aerobic		10.25	7.81	8.42	5.40	6.95
11	V7	Cream	13	6.5	0 8	0.995	aerobic		4.75	3.03	3.29	2.40	2.63
11	V7	Skim milk	4	6.5	0.8	0.995	aerobic		37.80	29.37	22.45	11.50	17.58
11	V7	Skim milk	8	6.5	0.8	0.995	aerobic		9.81	7.81	8.42	5.40	6.95
11	V7	Skim milk	13	6.5	0.8	0.995	aerobic		4.88	3.03	3.29	2.40	2.63
11		Whole milk	4	6.4	0.8	0.995			33.27	29.37	22.71	11.8	18.04
11		Whole milk	8	6.4	0.8	0.995			13.06	7.81	8.40	5.50	7.13
11		Whole milk	13	6.4	0.8	0.995			5.82	3.03	3.23	2.40	2.70
11		Whole milk	21	6.4	0.8	0.995			1.86	1.18	1.30	0.80	0.88
11		Whole milk	35	6.4	0.8	0.995			0.69	0.43	1.28	0.30	0.46
11		Skim milk	4	6.5	0.8	0.995			34.52	29.37	22.45	11.50	17.58
11		Skim milk	8	6.5	0.8	0.995			12.49	7.81	8.42	5.40	6.95
11		Skim milk	13	6.5	0.8	0.995			6.03	3.03	3.29	2.40	2.63
11		Skim milk	21	6.5	0.8	0.995			1.92	1.18	1.34	0.80	0.90
11		Skim milk	35	6.5	0.8	0.995			0.69	0.43	1.32	0.30	0.50
11		Chocolate milk	4	6.5	2.5	0.986			33.46	33.77	21.10	13.50	17.51
11		Chocolate milk	8	6.5	2.5	0.986			10.56	8.98	8.02	6.40	7.00

Table D3 - Predictive Model evaluation - Literature data													
Ref	Strain	Food	Temp	pH	%NaCl	aw	Atm	Assumptions	observed GT	Ross	Murphy	PMP	FMM
11		Chocolate milk	13	6.5	2.5	0.986			5.16	3.49	3.18	2.80	2.69
11		Chocolate milk	21	6.5	2.5	0.986			1.72	1.36	1.33	1.00	0.90
11		Chocolate milk	35	6.5	2.5	0.986			0.68	0.49	1.37	0.3	0.49
11		Whipping cream	4	6.5	0.8	0.995			36.30	29.37	22.45	11.50	17.58
11		Whipping cream	8	6.5	0.8	0.995			11.93	7.81	8.42	5.40	6.95
11		Whipping cream	13	6.5	0.8	0.995			5.56	3.03	3.29	2.40	2.63
11		Whipping cream	21	6.5	0.8	0.995			1.80	1.18	1.34	0.80	0.90
11		Whipping cream	35	6.5	0.8	0.995			0.68	0.43	1.32	0.30	0.50
11	California	Skim milk	4	6.5	0.8	0.995			21.56	29.37	22.45	11.80	17.58
11	California	Whole milk	4	6.4	0.8	0.995			20.98	29.37	22.71	12.10	18.04
11	California	Chocolate milk	4	6.5	2.5	0.986			21.29	33.77	21.10	13.50	17.51
11	California	Cream	4	6.5	0.8	0.995			22.83	29.37	22.45	11.80	17.58
11	California	Skim milk	8	6.5	0.8	0.995			9.17	7.81	8.42	5.50	6.95
11	California	Whole milk	8	6.4	0.8	0.995			10.73	7.81	8.40	5.70	7.13
11	California	Chocolate milk	8	6.5	2.5	0.986			8.34	8.98	8.02	6.40	7.00
11	California	Cream	8	6.5	0.8	0.995			8.32	7.81	8 42	5.50	6.95
11	Scott A	Skim milk	4	6.4	0.8	0.995			32.50	29.37	22.71	12.10	17.58
11	Scott A	Skim milk	8	6.4	0.8	0.995			12.50	7.81	8.40	5.70	7.13
11	Scott A	Skim milk	13	6.4	0.8	0.995			6.00	3.03	3.23	2.50	2.70
11	V7	Skim milk	21	6.4	0.8	0.995			1.90	1.18	1.30	0.90	0.88
11	V7	Skim milk	35	6.4	0.8	0.995			0.70	0.43	1.28	0.30	0.46
12	V7	2% fat milk	13	6.5	0.8	0.995		aw	3.41	3.03	3.29	2.40	2.63
12	V7	2% fat milk	13	6.5	0.8	0.995		aw	2.86	3.03	3.29	2.40	2.63
12	V7	2% fat milk	13	6.5	0.8	0.995		aw	3.24	3.03	3.29	2.40	2.63
12	V7	2% fat milk	13	6.5	0.8	0.995		aw	2.78	3.03	3.29	2.40	2.63
12	2 x clinical/food	2% fat milk	13	6.5	0.8	0.995		aw	4.48	3.03	3.29	2.40	2.63
12	3 x clinical/food	2% fat milk	13	6.5	0.8	0.995		aw	4.37	3.03	3.29	2.40	2.63
12	4 x clinical/food	2% fat milk	13	6.5	0.8	0.995		aw	4.88	3.03	3.29	2.40	2.63
12	5 x clinical/food	2% fat milk	13	6.5	0.8	0.995		aw	3.92	3.03	3.29	2.40	2.63

Table D3 - Predictive Model evaluation - Literature data													
Ref	Strain	Food	Temp	pH	%NaCl	aw	Atm	Assumptions	observed GT	Ross	Murphy	PMP	FMM
13	Scott A	Camembert	6	6.1	2.5	0.986	aerobic		50.70	18.25	13.55	10.40	12.32
13	OH	Camembert	6	6.1	2.5	0.986	aerobic		21.69	18.25	13.55	10.40	12.32
13	OH	Camembert	6	6.1	2.5	0.986	aerobic		18.00	18.25	13.55	10.40	12.32
14	Scott A	Uncultured whey	6	5.6	0.8	0.995	aerobic		28.90	95.20	19.08	13.90	16.29
14	Scott A	Uncultured whey	6	6.2	0.8	0.995	aerobic		21.10	13.60	13.80	9.00	11.29
14	Scott A	Uncultured whey	6	6.8	0.8	0.995	aerobic		18.00	13.60	13.52	7.60	10.34
14	Scott A	Cultured whey	6	5.6	0.8	0.995	aerobic		19.40	95.20	19.08	13.90	16.29
14	Scott A	Cultured whey	6	6.2	0.8	0.995	aerobic		10.30	13.60	13.80	9.00	11.29
14	Scott A	Cultured whey	6	6.8	0.8	0.995	aerobic		9.50	13.60	13.52	7.60	10.34
14	OH	Uncultured whey	6	5.6	0.8	0.995	aerobic		25.20	95.20	19 08	13.90	16.29
14	V7	Uncultured whey	6	5.6	0.8	0.995	aerobic		31.60	95.20	19.08	13.90	16.29
14	V7	Uncultured whey	6	6.2	0.8	0.995	aerobic		14.80	13.60	13.80	9.00	11.29
14	V7	Uncultured whey	6	6.8	0.8	0.995	aerobic		14.00	13.60	13.52	7.60	10.34
14	OH	Cultured whey	6	5.6	0.8	0.995	aerobic		16.50	95.20	19.08	13.90	16.29
14	OH	Cultured whey	6	6.8	0.8	0.995	aerobic		7.30	13.60	13.52	7.60	10.34
15	4b - 433	UHT milk	0	6.6	0.5	0 997	aerobic		62.00	7662.20			
15	4b - 433	UHT milk	2.5	6.6	0.5	0.997	aerobic		24.00	68.00			25.70
15	4b - 433	UHT milk	5	6.6	0.5	0.997	aerobic		20.00	18.72	17.64	9.10	13.70
15	4b - 433	UHT milk	7.5	6.6	0.5	0.997	aerobic		16.00	8.60	9.71	5.80	7.69
15	4b - 433	UHT milk	9.3	6.6	0.5	0.997	aerobic		5.50	5.67	6.64	4.20	5.25
15	4b - 433	UHT milk	0	6.6	0.5	0.997	aerobic		77.00	7662.20			
15	4b - 433	UHT milk	2.5	6.6	0.5	0.997	aerobic		33.00	68.00			25.70
15	4b - 433	UHT milk	5	6.6	0.5	0.997	aerobic		19.00	18.72	17.64	9.10	13.70
15	4b - 433	UHT milk	7.5	6.6	0.5	0.997	aerobic		9.50	8.60	9.71	5.80	7.69
15	4b - 433	UHT milk	9.3	6.6	0.5	0.997	aerobic		9.00	5.67	6.64	4.20	5.25

References

1 Donnelly & Briggs (1986); 2 Farber *et al*. (1990b); 3 Ferguson & Shelef (1990);4 Marshall & Schmidt, (1988); 5 Murphy *et al*. (1996);
6 Northolt *et al*. (1988); 7 Papageorgiou & Marth (1989b); 8 Papageorgiou *et al*. (1996); 9 Pearson & Marth (1990); 10 Rajkowski *et al*. (1994);
11 Rosenow & Marth (1987a); 12 Rosenow & Marth (1987b); 13 Ryser & Marth (1987a); 14 Ryser & Marth (1988b); 15 Walker *et al*. (1990)

Appendix E – Product Case Study 1 – 1 kg Brie

Risk Assessment Results

Table E.1 – Brie measured process parameter distributions selected by Bestfit software and goodness of fit statistics

Process Step	Function	Goodness of fit	
Brie Production		**Chi-Square**	**Kolmogorov-Smirnov**
Temperature	Normal	1.504983	0.025667
pH	Normal	44.793874	0.041372
Salt concentration	Normal	2.747055	0.03282
Brie Draining			
Temperature	Normal	3.637971	0.039895
pH	Normal	12.039615	0.027265
Salt concentration	Normal	1.20706	0.020078
Brie Maturing			
Temperature	Normal	1.617495 e12	0.096319
pH	Normal	765.738274	0.06503
Salt concentration	Normal	2.472564	0.04629
Final product specifications			
pH	Normal	0.133041	0.022285
Salt concentration	Normal	0.198543	0.051377
Storage and transport			
Temperature	Normal	1.12435 e4	0.027725
Shelf life at 5°C			
pH	Normal	0.138318	0.066554
Salt concentration	Normal	5.186633	0.065095
Shelf life at 10°C			
pH	Normal	0.264903	0.043284
Salt concentration	Normal	0.435016	0.027545

Table E.2 – Simple risk assessment input variable ranges

	Minimum	Mean	Maximum
Temperature	-3.8134	0.00002	3.8999
pH	-3.8373	0.000006	3.8956
%NaCl	-3.7084	0.00005	4.1197

Table E.3 - Output values of predicted *L. monocytogenes* growth (log cfu/g) for Brie production process

	Minimum	Mean	Maximum	50th percentile	95th percentile
0 - 4 hr	0.4304	0.8298	1.0956	0.8340	0.9710
4 - 8 hr	0.5822	0.8665	1.0221	0.8711	0.9433
8 - 12 hr	0.3963	0.6986	0.8535	0.6451	0.7277
12 - 16 hr	0.0892	0.4430	0.6986	0.4487	0.5759
16 - 20 hr	0.1748	0.2735	0.4126	0.2725	0.3294
20 – 24 hr	0.0365	0.1226	0.3087	0.1188	0.1915
Total growth	2.4657	3.1793	3.8105	3.1871	3.4603

Table E.4 - Correlation of output values with Brie production process parameter inputs values

	Temperature	pH	%NaCl
0 - 4 hr	-0.9763	0.0691	-0.0510
4 - 8 hr	-0.5696	0.7466	-0.1897
8 - 12 hr	0.5710	0.7637	-0.1383
12 - 16 hr	0.9363	0.3197	-0.0575
16 - 20 hr	0.5936	0.7218	-0.2838
20 - 24 hr	0.4969	0.4391	-0.7130
Total growth	0.1980	0.8691	-0.3525

Table E.5 - Output values of predicted *L. monocytogenes* growth (log cfu/g) for Brie draining process

	Minimum	Mean	Maximum	50th percentile	95th percentile
0 - 4 hr	0.0035	0.0525	0.1761	0.0498	0.0938
4 - 8 hr	0.0014	0.0362	0.1350	0.0334	0.0709
8 - 12 hr	0.0004	0.0370	0.1410	0.0330	0.0798
12 - 16 hr	0.0019	0.0511	0.1586	0.0478	0.0966
16 - 20 hr	0.0079	0.0642	0.1635	0.0622	0.1055
20 – 24 hr	0.0297	0.0726	0.1592	0.0714	0.1045
Total growth	0.0381	0.3197	0.9165	0.2986	0.5487

Table E.6 - Correlation of output values with Brie draining process parameter inputs values

	Temperature	pH	%NaCl
0 - 4 hr	-0.1618	0.3417	-0.9082
4 - 8 hr	0.1898	0.1840	-0.9537
8 - 12 hr	0.1467	0.0555	-0.9824
12 - 16 hr	0.1701	0.0488	-0.9779
16 - 20 hr	0.1974	0.1575	-0.9574
20 - 24 hr	0.2781	0.3240	-0.8815
Total growth	0.1901	0.1808	-0.9547

Table E.7 - Output values of predicted *L. monocytogenes* growth (log cfu/g) for Brie maturation process

	Minimum	Mean	Maximum	50th percentile	95th percentile
Day 1	0.1785	0.5838	1.2337	0.5726	0.8196
Day 2	0.1542	0.5586	1.2521	0.5474	0.8005
Day 3	0.1383	0.5490	1.2752	0.5386	0.7986
Day 4	0.2245	0.6454	1.3529	0.6344	0.8944
Day 5	0.1694	0.8011	1.6664	0.7886	1.1469
Day 6	0.0867	1.0508	2.1736	1.0557	1.5337
Day 7	0.4104	1.5310	3.2326	1.4973	2.1118
Total	1.3752	5.7196	11.65	5.6327	7.9451

Table E.8 - Correlation of output values with Brie maturation process input values

	Temperature	pH	%NaCl
Day 1	0.6253	0.5629	-0.4853
Day 2	0.5827	0.5040	-0.5904
Day 3	0.4569	0.2976	-0.8138
Day 4	0.6657	0.3634	-0.6126
Day 5	0.4518	0.7659	-0.3967
Day 6	0.4876	0.7876	-0.2822
Day 7	0.9279	0.1622	-0.2651
Total	0.6694	0.5251	-0.4636

Table E.9 - Output values of predicted *L. monocytogenes* growth (log cfu/g) for Brie storage and distribution process

	Minimum	Mean	Maximum	50th percentile	95th percentile
Day 1	0.0228	0.2303	0.9685	0.2102	0.4334
Day 2	0.0435	0.1988	0.7048	0.1869	0.3358
Day 3	0.0536	0.1563	0.3407	0.1533	0.2207
Day 4	0.0422	0.1998	0.6894	0.1886	0.3350
Total	0.1807	0.7852	2.7034	0.7405	1.3095

Table E.10 - Correlation of output values with Brie storage and distribution process input values

	Temperature	pH	%NaCl
Day 1	0.9808	0.0625	-0.1595
Day 2	0.9309	0.1189	-0.3028
Day 3	0.6529	0.2758	-0.6452
Day 4	0.7965	0.2038	-0.5064
Total	0.9034	0.1452	-0.3535

Table E.11 - Output values - Log growth for each stage of Brie shelf life, and overall total amount of growth possible

	Minimum	Mean	Maximum	50th percentile	95th percentile
Day 0-5	1.17	1.97	2.99	1.95	2.38
Day 5-10	1.35	2.16	3.24	2.15	2.60
Day 10-15	1.38	2.24	3.43	2.23	2.71
Day 15-20	1.34	2.17	3.24	2.16	2.60
Day 20-25	1.28	2.20	3.32	2.19	2.64
Day 25-30	1.28	2.19	3.37	2.17	2.64
Day 30-35	1.46	2.34	3.47	2.33	2.79
Day 35-40	1.42	2.30	3.41	2.28	2.75
Day 40-45	1.33	2.23	3.32	2.22	2.67
Day 45-50	1.37	2.32	3.47	2.31	2.77
Day 50-55	1.39	2.31	3.43	2.30	2.76
Total	14.79	24.43	36.61	24.29	29.29

Table E.12 - Correlation of output values with Brie shelf life process input values

	Temperature	pH	%NaCl
Day 0-5	0.9781	0.1364	-0.1912
Day 5-10	0.9802	0.0404	-0.1815
Day 10-15	0.9198	0.0224	-0.3551
Day 15-20	0.9825	0.0239	-0.1708
Day 20-25	0.9716	0.0705	-0.1923
Day 25-30	0.9400	0.0999	-0.2870
Day 30-35	0.9877	0.0199	-0.1419
Day 35-40	0.9761	0.0228	-0.1968
Day 40-45	0.9817	0.0530	-0.1580
Day 45-50	0.9571	0.0567	-0.1200
Day 50-55	0.9768	0.0358	-0.1873

Table E13 - Brie detailed risk assessment intputs

Name	Contamination level	Consumption time	Production Temperature	Production pH	Production %NaCl	Contamination time	Serving size	Lag phase	Contamination frequency	Draining Temperature
Minimum =	-2.974645	365.4246	-3.950408	-4.18294	-3.813146	8.18E-03	5.074276	0.3115923	3.80E-05	-3.7269
Maximum =	2 954527	1413.337	3.764435	4.222005	4.461296	215.9849	124.0659	55.97638	4.28E-02	3.825295
Mean =	-0.3333353	727.3337	-5.10E-06	-4.34E-06	5.68E-05	108	53 33321	4.999236	1.53E-02	3.11E-06
Std Deviation =	1.247225	244.2988	0.9998928	1.000098	1.000078	62.35382	25.84682	3.859168	9.80E-03	0.9998817
Variance =	1.55557	59681.91	0.9997855	1.000195	1.000157	3887.998	668.0581	14.89318	9.61E-05	0.9997635
Skewness =	0.305416	0 5634114	-3.98E-04	5.11E-05	2 85E-03	8.02E-07	0.4680553	2.67815	0.5558234	1.95E-04
5% Perc =	-2.225718	408.3539	-1.644861	-1.645708	-1.644977	10.79547	17.23987	1.280846	2.54E-03	-1.645292
10% Perc =	-1.905009	435.2848	-1.282081	-1.281821	-1.28204	21.5916	22.31574	1.642796	3.65E-03	-1.281873
15% Perc =	-1.658466	462.9702	-1.036453	-1.03652	-1.036761	32.3832	26.20749	1.943737	4.76E-03	-1.036525
20% Perc =	-1 45101	491.4727	-0.8416221	-0.8419561	-0.8417434	43.19966	29.49463	2.22097	5.90E-03	-0.8419039
25% Perc =	-1 268203	520.8769	-0.6745503	-0.6745424	-0.6747181	53.98157	32.52979	2.490386	7.08E-03	-0.6745313
30% Perc =	-1.102915	551.3478	-0.5245695	-0.5245892	-0.5245357	64.79837	35.6635	2.760315	8.30E-03	-0.5246456
35% Perc =	-0.9496857	582.8323	-0.3853549	-0.3853936	-0 3855658	75.58379	38.91583	3.03611	9.56E-03	-0.3854828
40% Perc =	-0.7948468	615.5981	-0.2535552	-0.2533524	-0.2535658	86.38033	42.29502	3.323866	1.09E-02	-0.2535188
45% Perc =	-0.6331862	649.7814	-0.1257899	-0.1258062	-0.1257309	97.18052	45.81042	3.627806	1.22E-02	-0.1256711
50% Perc =	-0.4643007	685.509	-2.60E-05	-4.45E-05	-1.80E-04	107.9903	49.49453	3.953719	0.0136721	-9.84E-05
55% Perc =	-0.2863608	723.1253	0.125557	0.1254096	0.1254818	118.7868	53.37448	4.309434	1.52E-02	0.1254272
60% Perc =	-9.87E-02	762.8363	0.2532558	0.2533198	0.2532561	129.5936	57.47087	4.703235	1.68E-02	0.2530974
65% Perc =	0 1014895	805.1376	0.3851302	0.3852556	0.3851353	140.3924	61.82864	5.148028	1.85E-02	0.3851221
70% Perc =	0.3164737	850.5756	0.5242936	0.5242262	0.5242775	151.1961	66.51409	5.663245	0.0202812	0.5243562
75% Perc =	0 5500236	900.0569	0.6744385	0.674425	0.6744762	161.9834	71.60506	6.276124	2.23E-02	0.6743901
80% Perc =	0.808943	954 6582	0.8415771	0 8412704	0.8414247	172.7934	77.2439	7.037075	2.45E-02	0.8416161
85% Perc =	1.102199	1016.756	1.036309	1.036051	1.036189	183.5907	83.63851	8.04153	0.0269369	1.036233
90% Perc =	1.450106	1090.278	1.281404	1.28149	1 281086	194.3944	91.22705	9.51242	2.99E-02	1.281466
95% Perc =	1.903471	1186.197	1.644508	1.644146	1.644173	205.1902	101.1101	12.19709	3.37E-02	1.644548

Table E13 - Brie detailed risk assessment intputs

Name	Draining pH	Draining %NaCl	Maturation Temperature	Maturation pH	Maturation %NaCl	Distribution Temperature	Distribution pH	Distribution %NaCl	Consumer Temperature	Consumer pH	Consumer %NaCl
Minimum =	-3.836925	-3.727182	-3.778842	-3.761958	-4.30018	-4.310652	-3.761449	-4.082033	-4.687216	-3.730248	-3.725355
Maximum =	3.710702	3.837327	4.180525	4.051442	4.041547	3.830197	4.114643	4 015594	3.883861	3.905915	4.060834
Mean =	-1.53E-06	2.23E-05	3.20E-05	1.66E-05	-2.60E-05	-5.78E-05	4.02E-05	-2.34E-06	-7.48E-05	3.47E-05	3.75E-05
Std Deviation =	0.9998072	0.9998056	0.9999775	0.9998937	1.00014	1.000119	0.999921	1.000011	1.000259	0.9998603	0.9998785
Variance =	0.9996145	0.9996111	0.9999549	0.9997873	1.000281	1.000237	0.9998419	1.000021	1.000518	0.9997208	0.9997571
Skewness =	-4.44E-05	8.22E-04	1.50E-03	9 00E-04	-1.26E-03	-2.57E-03	1.67E-03	-2.51E-04	-3.97E-03	1.24E-03	1.56E-03
5% Perc =	-1.645148	-1.645752	-1.645361	-1.644912	-1.645588	-1.645154	-1.645059	-1.645648	-1.644925	-1.645373	-1.645765
10% Perc =	-1 281958	-1.281731	-1.2818	-1.281802	-1.281568	-1 28158	-1.281656	-1.281768	-1.281853	-1 281844	-1.281706
15% Perc =	-1.036645	-1.0368	-1.036717	-1.036453	-1.036587	-1.036452	-1.036568	-1.036681	-1.036528	-1.036705	-1.036774
20% Perc =	-0.8419698	-0.8418314	-0.8416877	-0.8417864	-0.841636	-0.8418446	-0.8418201	-0.8416633	-0.8419561	-0.841765	-0.8417385
25% Perc =	-0.6745571	-0.6744833	-0.6747444	-0.6746458	-0.6747819	-0.6746735	-0 6746822	-0.6745234	-0.6744986	-0.6747373	-0.6746442
30% Perc =	-0 5245396	-0.5244945	-0.5244154	-0.5244051	-0.5244312	-0.5246509	-0 5244571	-0.5244531	-0.5245293	-0.5244451	-0.5244091
35% Perc =	-0 3854393	-0.3854699	-0.3854881	-0.3854545	-0.3853591	-0.3853835	-0 3854018	-0.3854056	-0.3855251	-0.385337	-0.385527
40% Perc =	-0.2534311	-0.2535962	-0.2535858	-0.2533864	-0.2534613	-0.2533863	-0.2533858	-0 2533507	-0.2533714	-0.2535437	-0.2533979
45% Perc =	-0 1258075	-0.12584	-0.1257801	-0.1257456	-0.1258049	-0.1257319	-0.1257299	-0.1257965	-0.1257648	-0.1256964	-0.1258281
50% Perc =	-4.86E-05	-2.30E-04	-2.06E-04	-1.27E-04	-1.06E-05	-1.69E-04	-8.30E-06	-2.27E-04	-1.40E-04	-1.23E-04	-2.94E-05
55% Perc =	0.1255186	0.1255073	0.1255551	0.1255315	0.1256135	0.1255856	0.1254707	0.1256028	0 1256381	0.1255621	0.1255617
60% Perc =	0.2532966	0.2530901	0.2532015	0.253259	0.2532023	0.2532679	0.2532328	0.2533121	0.2531559	0.2531607	0.2531486
65% Perc =	0.3850792	0.3850675	0.3851502	0.3851597	0.3852851	0.3853066	0.3852541	0.3852955	0.3851304	0.3850684	0.3853057
70% Perc =	0.5241295	0.5243078	0.5241886	0.5243552	0.5241875	0.5241571	0.524394	0.5242922	0.5242462	0.5241628	0.524318
75% Perc =	0.6743603	0.6741729	0.674394	0.6741995	0.6743262	0.6742363	0.6742948	0.6743054	0.6744104	0.6744258	0.6742721
80% Perc =	0.8415473	0.841474	0.8413112	0.8413641	0.8412763	0.8415982	0.8415166	0.841339	0.8415552	0.8416013	0.8414012
85% Perc =	1.036248	1.036169	1.036195	1.036148	1.036109	1.036233	1.036154	1.036213	1.03614	1.036218	1.036289
90% Perc =	1.28115	1.28098	1.281477	1.28125	1.281088	1.28135	1.281056	1.28099	1.281369	1.28146	1.28099
95% Perc =	1.644017	1.644003	1.644509	1.644316	1.644553	1.644621	1.644384	1.644501	1 644273	1 644012	1.644065

Table E14 - Brie detailed risk assessment outputs, shelf life storage at 5 °C

Tasmania	Number of	Susceptible population								Susceptible population
Name	serves/annum	Cancer	Transplant	AIDS	Diabetes	Pregant	Kidney	< 30 days	> 60 years	Total
Minimum =	202652	8.76E-13	2.77E-12	2.19E-12	4.55E-12	5.86E-12	1.69E-13	6.40E-13	3 77E-12	2.08E-11
Maximum =	4954835	0.1986218	0.6268143	0.495575	1.031109	1.328063	3.84E-02	0.1449508	0 8543808	4.717907
Mean =	636739 9	5.13E-02	0.1619041	0.1280054	0.2663322	0.3430343	9.92E-03	3.74E-02	0.2206838	1.21862
Std Deviation =	437895.6	4.93E-02	0.1555737	0.1230005	0.2559188	0.3296219	9.53E-03	3.60E-02	0 2120552	1.170973
Variance =	1.92E+11	2.43E-03	2.42E-02	1.51E-02	6.55E-02	0.1086506	9.08E-05	1.29E-03	0.0449674	1.371177
Skewness =	2.688729	0.7875144	0.7875144	0.7875144	0.7875144	0.7875144	0.7875144	0.7875144	0.7875144	0.7875144
5% Perc =	248623.1	7.31E-07	2.31E-06	1.82E-06	3.80E-06	4.89E-06	1.41E-07	5.33E-07	3.14E-06	1 74E-05
10% Perc =	2.76E+05	1.53E-05	4.83E-05	3.82E-05	7.95E-05	1.02E-04	2.96E-06	1.12E-05	6 59E-05	3.64E-04
15% Perc =	3.01E+05	1.33E-04	4.21E-04	3.33E-04	6.92E-04	8.92E-04	2.58E-05	9.73E-05	5.74E-04	3.17E-03
20% Perc =	325429.6	9.21E-04	2.91E-03	2.30E-03	4.78E-03	6.16E-03	1.78E-04	6.72E-04	3.96E-03	2.19E-02
25% Perc =	3.51E+05	5.09E-03	1.61E-02	0.0127105	2.64E-02	3.41E-02	9.85E-04	3.72E-03	2.19E-02	0.1210048
30% Perc =	377931.8	1.14E-02	3.59E-02	2 84E-02	5.90E-02	7.61E-02	2.20E-03	8.30E-03	4 89E-02	0.2701781
35% Perc =	406592.1	1.77E-02	5.60E-02	4.42E-02	0.092043	0.1185508	3.43E-03	1.29E-02	7.63E-02	0.4211486
40% Perc =	437464.8	0.0241054	7.61E-02	6.01E-02	0.1251389	0.1611781	4.66E-03	1.76E-02	0.1036905	0.5725809
45% Perc =	471033.5	0.031009	9.79E-02	0.0773696	0.1609777	0.2073383	5.99E-03	2.26E-02	0.1333867	0.7365637
50% Perc =	507829.9	0.0383918	0.1211576	0.0957902	0.1993042	0.2567026	7.42E-03	2.80E-02	0.1651441	0.9119291
55% Perc =	548690.1	0.0460516	0.1453305	0.1149019	0.2390687	0.307919	8.90E-03	3.36E-02	0.1980931	1.093874
60% Perc =	5 94E+05	5.47E-02	0.1726776	0.1365232	0.2840546	0.3658607	0.0105765	0 0399317	0.2353686	1.29971
65% Perc =	645909.9	0.064188	0.2025657	0.1601535	0.3332205	0.429186	1.24E-02	4.68E-02	0.2761076	1.524672
70% Perc =	7.05E+05	7.46E-02	0.2355561	0.1862366	0.3874898	0.4990845	1.44E-02	5.45E-02	0.3210754	1.772984
75% Perc =	7.73E+05	8.55E-02	0.2698404	0.2133425	0.4438874	0.5717243	1.65E-02	6 24E-02	0.3678066	2.031035
80% Perc =	852381.2	9.72E-02	0.3068534	0.2426059	0.5047738	0.6501455	1.88E-02	7.10E-02	0.4182573	2.309625
85% Perc =	959106.8	0.1106331	0.3491379	0.2760371	0.5743318	0.7397358	2.14E-02	8.07E-02	0.4758933	2.627892
90% Perc =	1126209	0.1269748	0.4007095	0.316811	0.6591672	0.8490033	2.45E-02	9 27E-02	0.5461881	3.016062
95% Perc =	1456906	0.1479386	0.4668672	0.3691169	0.7679966	0.989175	2.86E-02	0.1079631	0.6363646	3.514018

Table E14 - Brie detailed risk assessment outputs, shelf life storage at 5 °C

Tasmania	Level at end	Level at time		General population						General population
Name	of storage	of consumption	Dose	1 - 9 years	10 - 19 years	20 - 29 years	30 - 39 years	40 - 49 years	50 - 59 years	Total
Minimum =	-2.81591	8.47E-03	0.7271171	1.21E-13	1.40E-13	4 53E-13	1.28E-12	6.79E-13	9 67E-13	3.63E-12
Maximum =	8	1.00E+08	1 24E+10	2.74E-02	3.18E-02	0.1027191	0.2890323	0.1537967	0 2190843	0.823773
Mean =	3.016091	7.19E+07	3 83E+09	7.07E-03	8.21E-03	0.026532	0.0746561	3.97E-02	5.66E-02	0.2127779
Std Deviation =	2.470254	4.34E+07	3.19E+09	6.79E-03	7.89E-03	2.55E-02	0.0717371	3.82E-02	5.44E-02	0.2044584
Variance =	6 102157	1.88E+15	1.02E+19	4 61E-05	6.22E-05	6 50E-04	5.15E-03	1.46E-03	2 96E-03	4.18E-02
Skewness =	0.1136423	-0.9464227	0.4687371	0.7875144	0.7875144	0.7875144	0.7875144	0.7875144	0.7875144	0.7875144
5% Perc =	-0.9416238	1477.362	68352 48	1.01E-07	1.17E-07	3.78E-07	1.06E-06	5.66E-07	8 06E-07	3.03E-06
10% Perc =	-0.2351578	2.87E+04	1.28E+06	2 11E-06	2.45E-06	7.92E-06	2.23E-05	1.19E-05	1 69E-05	6.35E-05
15% Perc =	0.2851574	260056.1	1.25E+07	1 84E-05	2.13E-05	6.90E-05	1.94E-04	1.03E-04	1.47E-04	5.53E-04
20% Perc =	0.7637811	1652455	8.04E+07	1.27E-04	1.47E-04	4.76E-04	1.34E-03	7.13E-04	1.02E-03	3.82E-03
25% Perc =	1.166885	1.19E+07	5.72E+08	7.02E-04	8.15E-04	2.63E-03	7.41E-03	3.94E-03	5.62E-03	2.11E-02
30% Perc =	1.554464	7.39E+07	1.50E+09	1.57E-03	1.82E-03	5.88E-03	1 66E-02	8.81E-03	1.25E-02	4.72E-02
35% Perc =	1.931087	1.00E+08	2.13E+09	2 44E-03	2.84E-03	9.17E-03	2.58E-02	1.37E-02	1.96E-02	7.35E-02
40% Perc =	2.276271	1.00E+08	2.64E+09	3.32E-03	3.86E-03	1.25E-02	3.51E-02	1.87E-02	0 0265888	1.00E-01
45% Perc =	2.596744	1.00E+08	3.07E+09	4 27E-03	4 96E-03	1.60E-02	4.51E-02	2.40E-02	3.42E-02	0.1286082
50% Perc =	2.952056	1.00E+08	3.51E+09	5.29E-03	6.14E-03	1.99E-02	5.59E-02	2.97E-02	4.23E-02	0.1592279
55% Perc =	3.289004	1.00E+08	3.94E+09	6 34E-03	7.37E-03	2.38E-02	0.0670138	3.57E-02	5 08E-02	0.1909965
60% Perc =	3.629084	1.00E+08	4.42E+09	7.54E-03	8.76E-03	2.83E-02	7.96E-02	4.24E-02	6.04E-02	0.2269367
65% Perc =	3 974419	1.00E+08	4.94E+09	8.84E-03	1.03E-02	3.32E-02	9.34E-02	4.97E-02	0 0708008	0.2662162
70% Perc =	4 359321	1.00E+08	5.51E+09	1 03E-02	1.19E-02	3 86E-02	0.108618	5.78E-02	8.23E-02	0.309573
75% Perc =	4.756367	1.00E+08	6.13E+09	1.18E-02	1.37E-02	4.42E-02	0.124427	6.62E-02	9.43E-02	0.3546301
80% Perc =	5.198893	1.00E+08	6.81E+09	1.34E-02	1.56E-02	5.03E-02	0.1414941	0.0752903	0.1072515	0.4032734
85% Perc =	5 769507	1.00E+08	7.58E+09	0.015238	1.77E-02	0.0572149	0.1609921	8.57E-02	0.1220307	0.4588446
90% Perc =	6.460498	1.00E+08	8.48E+09	1.75E-02	2.03E-02	6.57E-02	0.1847724	9.83E-02	0 1400561	0.5266212
95% Perc =	7 495786	1.00E+08	9.67E+09	2 04E-02	2.37E-02	7.65E-02	0.2152786	0.1145517	0.1631795	0.6135672

Table E14 - Brie detailed risk assessment outputs, shelf life storage at 5 °C

rest of Australia	Number of	Susceptible population								Susceptible population
Name	serves/annum	Cancer	Transplant	AIDS	Diabetes	Pregant	Kidney	< 30 days	> 60 years	Total
Minimum =	802950.8	3.47E-12	1.10E-11	8.66E-12	1.80E-11	2.32E-11	6.71E-13	2.53E-12	1.49E-11	8.25E-11
Maximum =	1.96E+07	0.7869823	2.483573	1.963575	4.085478	5.262071	0.1521189	0.5743263	3.385241	18.69337
Mean =	2522901	0.203275	0.6414989	0.507185	1.055266	1.359176	3.93E-02	0.1483466	0.8743967	4.828435
Std Deviation =	1735037	0.195327	0.6164167	0.4873545	1.014006	1.306033	3.78E-02	0.1425464	0.8402084	4.639647
Variance =	3.01E+12	3.82E-02	0.3799696	0.2375144	1.028207	1.705722	1.43E-03	2.03E-02	0.7059501	21 52632
Skewness =	2.688729	0.7875144	0.7875144	0.7875144	0.7875144	0.7875144	0.7875144	0.7875144	0.7875144	0.7875144
5% Perc =	985098.2	2.90E-06	9 14E-06	7.23E-06	1.50E-05	1.94E-05	5.60E-07	2.11E-06	1.25E-05	6.88E-05
10% Perc =	1091864	6.07E-05	1.92E-04	1.51E-04	3.15E-04	4.06E-04	1.17E-05	4.43E-05	2 61E-04	1.44E-03
15% Perc =	1190888	5.28E-04	1 67E-03	1.32E-03	2.74E-03	3.53E-03	1.02E-04	3.86E-04	2.27E-03	0.0125489
20% Perc =	1289422	3.65E-03	1.15E-02	9.11E-03	1.89E-02	2.44E-02	7.05E-04	2.66E-03	1 57E-02	8.67E-02
25% Perc =	1390924	2.02E-02	6.37E-02	5.04E-02	0.1047843	0.1349615	3.90E-03	1.47E-02	8 68E-02	0.4794473
30% Perc =	1497447	4.51E-02	0.1422256	0.1124471	0 2339611	0.3013405	8.71E-03	3.29E-02	0.193861	1.070504
35% Perc =	1611005	7.03E-02	0.2216986	0.1752805	0.3646942	0.4697239	1.36E-02	0.0512678	0.3021868	1.668682
40% Perc =	1733330	9.55E-02	0.3014147	0.238306	0 4958272	0.6386224	1.85E-02	6.97E-02	0.4108441	2.268689
45% Perc =	1866336	0.1228644	0.3877376	0.306555	0.6378284	0 821519	2.37E-02	8.97E-02	0.5285067	2.918424
50% Perc =	2012132	0.1521166	0.4800524	0.3795414	0.7896862	1 017111	2.94E-02	0.1110121	0.6543366	3.61326
55% Perc =	2174028	0.1824664	0.5758308	0.4552662	0.9472415	1.220041	3.53E-02	0.1331609	0.7848876	4.334164
60% Perc =	2354987	0.2168014	0.6841859	0.5409344	1.125486	1.449619	4.19E-02	0.158218	0.9325813	5.149732
65% Perc =	2559234	0.2543267	0.8026088	0.6345626	1.320292	1.700527	4.92E-02	0.1856033	1.093998	6.041078
70% Perc =	2792427	0.2957471	0.9333242	0.7379094	1.535318	1.977481	0.0571661	0.2158312	1.27217	7.024947
75% Perc =	3061657	0.3387919	1.069166	0.8453092	1.758778	2.265295	0.0654864	0.2472446	1.457329	8.0474
80% Perc =	3377318	0 3852627	1.215819	0.961257	2.000023	2.576017	7.45E-02	0.2811582	1.657225	9.151231
85% Perc =	3800188	0.438352	1.38336	1.093719	2.275626	2.930993	8.47E-02	0.3199019	1.885592	10.41227
90% Perc =	4462282	0.5031016	1.587698	1.255273	2.611763	3.363934	9.72E-02	0.367155	2.164115	11.95029
95% Perc =	5772575	0.5861645	1.849829	1.462521	3.042968	3.919325	0.113302	0.4277729	2.521414	13.9233

Table E14 - Brie detailed risk assessment outputs, shelf life storage at 5 °C

rest of Australia	General population						General population	Probability of	Total using
Name	1 - 9 years	10 - 19 years	20 - 29 years	30 - 39 years	40 - 49 years	50 - 59 years	Total	infection (per meal)	Buchanan R-value
Minimum =	4.78E-13	5.56E-13	1.80E-12	5.05E-12	2.69E-12	3.83E-12	1.44E-11	1.35E-14	1.54011E-07
Maximum =	0.1083947	0.1259335	0.4069951	1.145208	0.6093757	0.8680591	3 263966	2.32E-04	32212.736
Mean =	0.027998	3.25E-02	0.1051255	0.2958036	0.1573998	0.2242168	0.8430719	7.17E-05	6830.001
Std Deviation =	0.0269033	3.13E-02	0.1010152	0.2842379	0.1512455	0.2154501	0.8101084	5.96E-05	6643.2204
Variance =	7 24E-04	9.77E-04	1.02E-02	8.08E-02	2.29E-02	4.64E-02	0.6562755	3.55E-09	32962160.6
Skewness =	0.7875144	0.7875144	0.7875144	0.7875144	0.7875144	0.7875144	0.7875144	0.4686455	1.7804206
5% Perc =	3.99E-07	4.64E-07	1.50E-06	4.21E-06	2.24E-06	3.19E-06	1.20E-05	1.28E-09	0.12859513
10% Perc =	8.36E-06	9.71E-06	3.14E-05	8.83E-05	4.70E-05	6.70E-05	2.52E-04	2 39E-08	2.694833
15% Perc =	7.28E-05	8.45E-05	2.73E-04	7.69E-04	4.09E-04	5.83E-04	2.19E-03	2.34E-07	23.404452
20% Perc =	5.03E-04	5.84E-04	1.89E-03	5.31E-03	2.83E-03	4.03E-03	1 51E-02	1.50E-06	160.09239
25% Perc =	2.78E-03	3.23E-03	1.04E-02	2.94E-02	1.56E-02	2.23E-02	8 37E-02	1.07E-05	785.5767
30% Perc =	6.21E-03	7.21E-03	2.33E-02	0.0655821	3.49E-02	4.97E-02	0.186916	2.81E-05	1575.1966
35% Perc =	9.68E-03	1.12E-02	3.63E-02	0.1022281	5.44E-02	7.75E-02	0.2913612	3.99E-05	2396.4738
40% Perc =	1.32E-02	1.53E-02	4.94E-02	0.1389863	7.40E-02	0.1053505	0.3961258	4.94E-05	3189.0155
45% Perc =	1.69E-02	1.97E-02	6 35E-02	0.1787909	9.51E-02	0.1355221	0.5095732	5.74E-05	4087.9423
50% Perc =	2.10E-02	2.43E-02	7.87E-02	0.2213585	0.1177868	0.167788	0.6308954	6.56E-05	5041 4625
55% Perc =	2.51E-02	0.0291984	9.44E-02	0.2655231	0.1412873	0.2012645	0.7567694	7.37E-05	6068.4988
60% Perc =	2.99E-02	3.47E-02	0.1121208	0.3154871	0.1678735	0.2391368	0.8991721	8.27E-05	7194.787
65% Perc =	3.50E-02	4.07E-02	0.1315274	0 3700935	0.1969301	0.280528	1.054806	9 23E-05	8416.205
70% Perc =	4.07E-02	4.73E-02	0.1529483	0.430368	0 2290027	0.3262157	1.226595	1.03E-04	9673.537
75% Perc =	4.67E-02	5.42E-02	0.1752093	0.4930064	0.2623332	0.373695	1.405121	1.15E-04	11216.289
80% Perc =	5.31E-02	6.17E-02	0.1992421	0.5606302	0.2983164	0 4249534	1.597856	1.27E-04	12778.523
85% Perc =	6.04E-02	7.01E-02	0.2266978	0.6378853	0.3394245	0 4835121	1.818041	1.42E-04	14659.912
90% Perc =	6.93E-02	0.0805067	0.2601836	0.7321082	0.3895614	0.5549324	2.086587	1.59E-04	16837.714
95% Perc =	8 07E-02	9.38E-02	0.3031403	0.8529804	0.4538786	0.6465526	2.431085	1.81E-04	19919.983

Table E15 - Brie detailed risk assessment, shelf life storage at 10 °C

Tasmania	Susceptible population								Susceptible population
Name	Cancer	Transplant	AIDS	Diabetes	Pregant	Kidney	< 30 days	> 60 years	Total
Minimum =	2.43E-11	7.66E-11	6.06E-11	1 26E-10	1.62E-10	4.69E-12	1.77E-11	1.04E-10	5.76E-10
Maximum =	0.1982183	0.625541	0.4945683	1.029015	1.325365	3.83E-02	0.1446564	0.8526453	4.708323
Mean =	6.72E-02	0.212027	0.1676339	0.3487845	0.4492323	1.30E-02	4.90E-02	0.289004	1.595886
Std Deviation =	4.69E-02	0.1478772	0.1169154	0.243258	0.3133148	9.06E-03	0.0341966	0.2015644	1.113042
Variance =	2.20E-03	2.19E-02	1.37E-02	5.92E-02	9.82E-02	8.20E-05	1.17E-03	4.06E-02	1.238864
Skewness =	0.5603586	0.5603586	0.5603586	0.5603586	0.5603586	0.5603586	0.5603586	0.5603586	0.5603586
5% Perc =	4.80E-03	1.51E-02	1.20E-02	2.49E-02	3.21E-02	9.27E-04	3.50E-03	0.0206308	0.1139237
10% Perc =	1.21E-02	3.81E-02	3.01E-02	6 27E-02	8.08E-02	2.34E-03	8.82E-03	5 20E-02	0.2869558
15% Perc =	1.72E-02	5.43E-02	4.29E-02	8.94E-02	0.115095	3.33E-03	1.26E-02	7.40E-02	0.408872
20% Perc =	2.24E-02	0.0708401	5.60E-02	0.116532	0.1500925	4.34E-03	1.64E-02	9 66E-02	0.5331994
25% Perc =	2.79E-02	8.82E-02	6.97E-02	0.1450098	0.1867717	5.40E-03	2.04E-02	0.1201556	0.6635016
30% Perc =	3.37E-02	0.1063353	8.41E-02	0.1749216	0.225298	6.51E-03	2.46E-02	0.1449407	0.8003651
35% Perc =	3.96E-02	0.1249974	9.88E-02	0.2056207	0.2648382	7.66E-03	2.89E-02	0.170378	0.9408308
40% Perc =	4.57E-02	0.1442641	0.1140588	0.2373145	0.3056596	8.84E-03	3.34E-02	0.1966396	1.085847
45% Perc =	5.23E-02	0.1650175	0.130467	0.2714538	0.3496308	1.01E-02	0 0381603	0.2249275	1.242054
50% Perc =	5.91E-02	0.1864976	0.1474497	0.3067886	0.3951418	1.14E-02	4.31E-02	0.2542061	1.403731
55% Perc =	6.63E-02	0.2091388	0.1653504	0.3440333	0.4431129	1.28E-02	4.84E-02	0.2850672	1.574147
60% Perc =	7.40E-02	0.2333906	0.1845244	0.3839275	0.4944963	1.43E-02	5.40E-02	0.3181236	1.756685
65% Perc =	8.22E-02	0.2592995	0.2050086	0.4265476	0.5493907	1.59E-02	0.059963	0.3534388	1.951696
70% Perc =	9.08E-02	0.2863958	0.2264317	0.4711211	0.6068011	1.75E-02	6 62E-02	0.3903725	2.155644
75% Perc =	0.1001254	0.3159778	0.2498199	0.5197834	0.6694778	1.94E-02	7 31E-02	0.4306943	2.378302
80% Perc =	0.1106266	0.3491175	0.2760211	0.5742984	0.7396928	2.14E-02	8.07E-02	0.4758656	2.627739
85% Perc =	0.1223826	0.3862174	0.3053532	0.6353276	0.8182982	2.37E-02	8.93E-02	0.5264347	2.906982
90% Perc =	0.1369736	0 4322639	0.3417586	0.7110741	0.9158591	2 65E-02	1.00E-01	0.5891984	3.253565
95% Perc =	0.1553365	0.4902139	0.3875753	0.8064018	1.038641	0.0300256	0.113362	0.6681873	3.689743

Table E15 - Brie detailed risk assessment, shelf life storage at 10 °C

Tasmania	Level at	Level at end	Probability of	General population						General population
Name	consumption	of storage	infection (per meal)	1 - 9 years	10 - 19 years	20 - 29 years	30 - 39 years	40 - 49 years	50 - 59 years	Total
Minimum =	2.25E-02	-2.83539	1.30E-14	3.34E-12	3.88E-12	1.26E-11	3.53E-11	1.88E-11	2.68E-11	1.01E-10
Maximum =	1.00E+08	8	2.31E-04	2.73E-02	3.17E-02	0.1025104	0.2884452	0.1534843	0.2186392	0.8220997
Mean =	9.42E+07	3.016471	9 39E-05	9.25E-03	1.08E-02	3.47E-02	9.78E-02	5.20E-02	7.41E-02	0.2786506
Std Deviation =	2.22E+07	2.471136	5.18E-05	6.45E-03	7.50E-03	2 42E-02	6.82E-02	3.63E-02	5.17E-02	0.1943434
Variance =	4.91E+14	6.106512	2.68E-09	4.17E-05	5.62E-05	5.87E-04	4.65E-03	1.32E-03	2.67E-03	3.78E-02
Skewness =	-3.717529	9 24E-02	0.3323099	0.5603586	0.5603586	0.5603586	0 5603586	0.5603586	0.5603586	0.5603586
5% Perc =	1.96E+07	-0.9996542	1.36E-05	6.61E-04	7.67E-04	2.48E-03	6.98E-03	3.71E-03	5.29E-03	1.99E-02
10% Perc =	1.00E+08	-0.2605917	3.15E-05	1.66E-03	1.93E-03	6.25E-03	1.76E-02	9.35E-03	0.0133253	0.0501041
15% Perc =	1.00E+08	0.3245648	4.13E-05	2.37E-03	2.75E-03	8 90E-03	2.50E-02	1.33E-02	1.90E-02	7.14E-02
20% Perc =	1.00E+08	0.7699324	4.88E-05	3.09E-03	3.59E-03	1.16E-02	0.0326653	0.0173815	2 48E-02	9.31E-02
25% Perc =	1.00E+08	1.175884	5.51E-05	3.85E-03	4.47E-03	1 44E-02	4 06E-02	2.16E-02	3.08E-02	0.1158511
30% Perc =	1.00E+08	1.555822	6.12E-05	4.64E-03	5 39E-03	1.74E-02	4.90E-02	0.0260907	3.72E-02	0.1397482
35% Perc =	1.00E+08	1.913425	6.74E-05	5.46E-03	6.34E-03	2 05E-02	5 76E-02	3.07E-02	4.37E-02	0.1642743
40% Perc =	1.00E+08	2.279547	7.39E-05	6.30E-03	7.32E-03	2 36E-02	6.65E-02	0.035397	5.04E-02	0.1895951
45% Perc =	1.00E+08	2.628476	8.08E-05	7.20E-03	8.37E-03	2.70E-02	7.61E-02	4.05E-02	5.77E-02	0.2168696
50% Perc =	1.00E+08	2.948366	8.78E-05	8.14E-03	9.46E-03	3.06E-02	8.60E-02	4.58E-02	6 52E-02	0.2450993
55% Perc =	1.00E+08	3 314487	9.51E-05	9.13E-03	1.06E-02	3.43E-02	9.64E-02	5.13E-02	7.31E-02	0.2748548
60% Perc =	1.00E+08	3 644391	1.03E-04	1.02E-02	1.18E-02	3.82E-02	0.1076195	5.73E-02	8.16E-02	0.306727
65% Perc =	1.00E+08	3.978725	1.12E-04	1.13E-02	1.31E-02	4.25E-02	0.1195664	6.36E-02	0.0906304	0.340777
70% Perc =	1.00E+08	4 349729	1.21E-04	1.25E-02	1.45E-02	4.69E-02	0.1320609	7.03E-02	0.1001011	0.3763876
75% Perc =	1.00E+08	4.773809	1.30E-04	1.38E-02	1.60E-02	5.18E-02	0.1457015	7.75E-02	0.1104406	0.4152649
80% Perc =	1.00E+08	5 235298	1.41E-04	1.52E-02	1.77E-02	5.72E-02	0.1609827	8.57E-02	0.1220236	0.4588179
85% Perc =	1.00E+08	5.765515	1.53E-04	1.69E-02	1.96E-02	0.0632913	0.1780899	9.48E-02	0.1349908	0.5075754
90% Perc =	1.00E+08	6.438086	1.68E-04	1.89E-02	2.19E-02	7.08E-02	0.1993226	0.1060613	0.151085	0.5680906
95% Perc =	1.00E+08	7.398272	1.88E-04	2 14E-02	2.49E-02	0.0803337	0.2260441	0.1202801	0.1713397	0.6442498

Table E15 - Brie detailed risk assessment, shelf life storage at 10 °C

rest of Australia	Susceptible population								Susceptible population
Name	Cancer	Transplant	AIDS	Diabetes	Pregant	Kidney	< 30 days	> 60 years	Total
Minimum =	9.62E-11	3.03E-10	2.40E-10	4.99E-10	6.43E-10	1.86E-11	7.02E-11	4.14E-10	2.28E-09
Maximum =	0.7853836	2.478528	1.959586	4.077179	5.251382	0.1518099	0.5731597	3.378364	18.65539
Mean =	0.2662057	0.8400969	0.6642016	1.381959	1.779955	5.15E-02	0.1942724	1.145096	6.323243
Std Deviation =	0.1856638	0.5859213	0.4632441	0.9638406	1.241421	3.59E-02	0.1354943	0.7986416	4.410114
Variance =	3.45E-02	0.3433038	0.2145951	0.9289887	1.541126	1.29E-03	1.84E-02	0.6378284	19.44911
Skewness =	0.5603586	0.5603586	0.5603586	0.5603586	0.5603586	0.5603586	0.5603586	0.5603586	0.5603586
5% Perc =	1.90E-02	6.00E-02	4.74E-02	9.87E-02	0.1270636	3.67E-03	0.0138683	8.17E-02	0.4513902
10% Perc =	4.79E-02	0.1510576	0.1194299	0.2484898	0.3200533	9.25E-03	3.49E-02	0.2058995	1.136981
15% Perc =	6.82E-02	0.215236	0.170171	0.3540633	0.4560313	1.32E-02	4.97E-02	0.293378	1.620039
20% Perc =	8.89E-02	0.2806838	0.2219156	0.4617248	0.5946987	1.72E-02	6.49E-02	0.3825867	2.112651
25% Perc =	0.110677	0.3492766	0.2761468	0.57456	0.7400299	2.14E-02	8.08E-02	0.4760824	2.628936
30% Perc =	0.1335069	0.4213235	0.3331089	0.6930772	0.8926792	2.58E-02	9.74E-02	0.5742861	3.171219
35% Perc =	0.1569376	0.4952666	0.3915702	0.8147137	1.049346	3.03E-02	0.1145083	0.6750745	3.727774
40% Perc =	0.1811275	0.5716055	0.4519256	0.940291	1.211089	3.50E-02	0.1321838	0.7791284	4.302362
45% Perc =	0.2071839	0.6538349	0.5169383	1.075558	1.385313	4.00E-02	0.1511993	0.8912114	4.921287
50% Perc =	0.2341528	0.7389438	0.5842274	1.215563	1.565637	4.53E-02	0.1708807	1.007219	5.561884
55% Perc =	0.2625794	0.828653	0.6551538	1.363134	1.755709	0.050755	0.191626	1.129498	6.237108
60% Perc =	0.2930282	0.9247438	0.7311255	1.521204	1.959301	5.66E-02	0.213813	1.260474	6.960363
65% Perc =	0.3255575	1.0274	0.8122884	1.690074	2.176805	6.29E-02	0.2375863	1.400401	7.733039
70% Perc =	0.3595777	1.134762	0.8971711	1.866683	2.404277	6.95E-02	0.2624083	1.54674	8.541128
75% Perc =	0.3967186	1.251972	0.9898403	2.059494	2.652616	7.67E-02	0.2895185	1.706504	9.423346
80% Perc =	0.4383265	1.383279	1.093655	2.275494	2.930822	8.47E-02	0.3198833	1.885482	10.41167
85% Perc =	0.4849064	1.530277	1.209875	2.517305	3.242274	9.37E-02	0.3538765	2.085848	11.51809
90% Perc =	0 542719	1.712723	1.354121	2.817429	3.628831	0.1049043	0.3960671	2.334531	12.89132
95% Perc =	0 6154768	1.942333	1.535657	3.195138	4.115318	0.1189679	0.4491645	2.647502	14.61956

Table E15 - Brie detailed risk assessment, shelf life storage at 10 °C

rest of Australia	General population						General population
Name	1 - 9 years	10 - 19 years	20 - 29 years	30 - 39 years	40 - 49 years	50 - 59 years	Total
Minimum =	1.32E-11	1.54E-11	4 97E-11	1.40E-10	7.45E-11	1.06E-10	3.99E-10
Maximum =	0.1081745	0.1256777	0.4061683	1.142882	0.6081378	0.8662958	3.257336
Mean =	3.67E-02	4.26E-02	0.1376707	0.3873797	0.2061283	0.2936308	1.104074
Std Deviation =	2.56E-02	2.97E-02	9.60E-02	0.2701761	0.1437631	0.2047914	0.7700307
Variance =	6.54E-04	8.83E-04	9.22E-03	7.30E-02	2.07E-02	0 0419395	0.5929472
Skewness =	0.5603586	0.5603586	0.5603586	0.5603586	0.5603586	0 5603586	0.5603586
5% Perc =	2.62E-03	3.04E-03	9.83E-03	2.77E-02	1.47E-02	2 10E-02	7.88E-02
10% Perc =	6.59E-03	7.66E-03	2.48E-02	6.97E-02	3.71E-02	5.28E-02	0.1985232
15% Perc =	9.39E-03	1.09E-02	3 53E-02	9.92E-02	5.28E-02	7.52E-02	0.2828679
20% Perc =	1.23E-02	1.42E-02	4.60E-02	0.129427	6.89E-02	9.81E-02	0.3688807
25% Perc =	1.52E-02	1.77E-02	5.72E-02	0.1610561	8.57E-02	0.1220792	0.459027
30% Perc =	1.84E-02	2.14E-02	6.90E-02	0.1942778	0.103377	0.1472611	0.5537126
35% Perc =	2.16E-02	2.51E-02	8.12E-02	0.228374	0.1215199	0.1731057	0.6508903
40% Perc =	2.49E-02	2.90E-02	9.37E-02	0.2635748	0.1402505	0.1997877	0.7512165
45% Perc =	0 0285364	3.32E-02	0.1071471	0.3014919	0.1604266	0.2285285	0.8592842
50% Perc =	3.23E-02	3.75E-02	0.1210943	0.3407367	0.1813091	0.2582758	0.9711362
55% Perc =	3.62E-02	4.20E-02	0.1357954	0.3821028	0.2033204	0.289631	1.089034
60% Perc =	4.04E-02	4.69E-02	0.1515422	0.4264115	0.2268974	0.3232166	1.215318
65% Perc =	4.48E-02	5.21E-02	0.168365	0.4737478	0 2520855	0.3590972	1.350232
70% Perc =	4.95E-02	5 75E-02	0.1859589	0.5232536	0.278428	0.3966222	1.491329
75% Perc =	5.46E-02	6.35E-02	0.2051667	0.5773007	0.307187	0.4375895	1.645369
80% Perc =	6.04E-02	7.01E-02	0.2266846	0.6378481	0.3394048	0 483484	1.817936
85% Perc =	0.0667884	7.76E-02	0.2507738	0.7056307	0.3754725	0.5348626	2 011123
90% Perc =	7.48E-02	0.0868463	0.2806721	0.7897589	0.4202379	0 5986312	2.250898
95% Perc =	8.48E-02	9.85E-02	0.3182994	0.8956353	0.4765757	0.6788847	2.552657

Table E16 - Brie risk assessment, 30 day shelf life

Name	Probability of infection (per meal)	Level at time of consumption	Dose	Susceptible population Total in Tasmania	General population Total in Tasmania	Susceptible population Total in Australia	General population Total in Australia
Minimum =	5.88E-14	3.96E-02	3.148888	1.65E-11	2.87E-12	6.52E-11	1.14E-11
Maximum =	2.32E-04	1 00E+08	1 24E+10	0.3288941	5.74E-02	1.303149	0.2275372
Mean =	6.99E-05	6.97E+07	3.74E+09	7.70E-02	0.0134504	0.3052216	0.0532934
Std Deviation =	5.95E-05	4.40E+07	3.18E+09	8.07E-02	1.41E-02	0.319689	0.0558195
Variance =	3.54E-09	1.94E+15	1.01E+19	6.51E-03	1 98E-04	0.1022011	3 12E-03
Skewness =	0.4941527	-0.826147	0.4942416	0.9282286	0.9282286	0.9282286	0.9282286
5% Perc =	1.19E-09	1299.108	63798.82	9.18E-07	1.60E-07	3.64E-06	6.35E-07
10% Perc =	2.31E-08	29343.13	1234501	1.79E-05	3.12E-06	7.10E-05	1.24E-05
15% Perc =	2.04E-07	243022.9	1.09E+07	1.68E-04	2.94E-05	6.68E-04	1.17E-04
20% Perc =	1.27E-06	1514033	6 79E+07	9.67E-04	1.69E-04	3.83E-03	6.69E-04
25% Perc =	6.99E-06	8350372	3 74E+08	3.52E-03	6.15E-04	1.40E-02	2.44E-03
30% Perc =	2.25E-05	3 51E+07	1 20E+09	9.26E-03	1.62E-03	3.67E-02	6.41E-03
35% Perc =	3.56E-05	1.00E+08	1.91E+09	1.76E-02	3.07E-03	6.98E-02	1.22E-02
40% Perc =	4.63E-05	1.00E+08	2.48E+09	0.0269725	4.71E-03	0.1068811	1.87E-02
45% Perc =	5.49E-05	1.00E+08	2.93E+09	3 81E-02	6.65E-03	0.1510042	2.64E-02
50% Perc =	6.31E-05	1.00E+08	3.37E+09	5 07E-02	8 85E-03	0.2009056	3.51E-02
55% Perc =	7.23E-05	1.00E+08	3.86E+09	6.33E-02	1 10E-02	0.2506413	4.38E-02
60% Perc =	8.14E-05	1.00E+08	4.35E+09	7.80E-02	1.36E-02	0.3091124	5.40E-02
65% Perc =	9.07E-05	1.00E+08	4.85E+09	9.38E-02	1.64E-02	0.3716488	6.49E-02
70% Perc =	1.01E-04	1.00E+08	5.40E+09	0.1108674	1.94E-02	0.4393484	0.0767127
75% Perc =	1.13E-04	1.00E+08	6.03E+09	0.129932	2.27E-02	0.5149183	8.99E-02
80% Perc =	1.26E-04	1.00E+08	6.73E+09	0.1511233	2.64E-02	0.5989994	0 1045887
85% Perc =	1.40E-04	1.00E+08	7.50E+09	0.1752998	3.06E-02	0.6946712	0.1212935
90% Perc =	1.57E-04	1.00E+08	8.41E+09	0.2037973	3.56E-02	0.807714	0.1410314
95% Perc =	1.79E-04	1.00E+08	9.59E+09	0.2399639	4.19E-02	0.9511163	0.1660702

Table E17 - Brie risk assessment, contamination frequency (0, 0.3, 0.65) from Bemrah *et al* (1998)

Tasmania	Susceptible population								Susceptible population
Name	Cancer	Transplant	AIDS	Diabetes	Pregant	Kidney	≥ 60 years	< 30 days	Total
Minimum =	1.21E-10	3.82E-10	3.02E-10	6.29E-10	8.10E-10	2.34E-11	5.21E-10	8 84E-11	2.88E-09
Maximum =	3.004269	9.480928	7.495859	15 59613	20.08772	0.5807068	12.923	2 192465	71.36108
Mean =	1.052875	3.322683	2.626996	5.465813	7.039934	0.2035143	4.528991	0.7683704	25.00918
Std Deviation =	0 8211251	2.591322	2.048764	4.262724	5.490363	0.1587185	3.532108	0.5992432	19.50437
Variance =	0.6742465	6.714949	4.197433	18.17082	30.14409	2.52E-02	12.47579	0.3590924	380.4204
5% Perc =	2.23E-05	7.05E-05	5.57E-05	1.16E-04	1.49E-04	4.32E-06	9.60E-05	1.63E-05	5.30E-04
10% Perc =	4.23E-04	1.33E-03	1.05E-03	2.19E-03	2.83E-03	8.17E-05	1.82E-03	3.08E-04	1.00E-02
15% Perc =	3.77E-03	1.19E-02	9.39E-03	1.95E-02	2.52E-02	7.28E-04	1 62E-02	2 76E-03	8.94E-02
20% Perc =	2.51E-02	7.93E-02	0.062696	0.1304473	0.1680154	4.86E-03	0.1080891	1.83E-02	0.5968701
25% Perc =	0.1340377	0.4229988	0.3344334	0.695833	0.8962287	2.59E-02	0.5765696	9.78E-02	3 183828
30% Perc =	0.3794028	1.199037	0.9466362	1.969602	2 536835	7.33E-02	1.632019	0.2772773	9.012039
35% Perc =	0.6114641	1.931665	1 525645	3.174307	4.088488	0.1181923	2.630241	0.4466976	14.52424
40% Perc =	0.7948372	2.508362	1.983174	4.126256	5.314592	0.1536372	3.419029	0.5800588	18.87995
45% Perc =	0.9545912	3.012516	2.381771	4.95559	6.38277	0 1845166	4.106218	0.6966444	22.67462
50% Perc =	1.098511	3.466701	2.74086	5.702723	7.345073	0.2123354	4.725296	0.8016746	26.09317
55% Perc =	1.219792	3.849444	3.043466	6.332335	8.156009	0.2357784	5.246994	0.8901838	28.974
60% Perc =	1.333064	4.207113	3.326087	6.920364	8.913386	0.2576731	5.734237	0.9728948	31.66457
65% Perc =	1.442915	4.553578	3.600173	7.490636	9.647894	0.2789067	6.206767	1.053015	34.27388
70% Perc =	1.559299	4.922386	3.890559	8.094824	10.42608	0.301403	6.707398	1.138302	37.03838
75% Perc =	1.687672	5.325988	4.210859	8 76125	11.28444	0.3262168	7.259601	1.231635	40.08766
80% Perc =	1.827778	5.768136	4.560432	9.488584	12.22124	0.3532983	7.862273	1.333881	43.41562
85% Perc =	1.9888	6.276292	4.962193	10.3245	13 29789	0.3844229	8.554915	1.451393	47.24041
90% Perc =	2.177588	6.872074	5.433233	11.30456	14 56021	0.4209145	9.366998	1.589167	51.72474
95% Perc =	2.420323	7 6381	6.038872	12.56467	16.18322	0.4678336	10.41113	1.766311	57.49047

Table E17 - Brie risk assessment, contamination frequency (0, 0.3, 0.65) from Bemrah *et al* (1998)

Tasmania	General population						General population
Name	1 - 9 years	10 - 19 years	20 - 29 years	30 - 39 years	40 - 49 years	50 - 59 years	Total
Minimum =	1.67E-11	1.94E-11	6.26E-11	1.76E-10	9.38E-11	1.34E-10	5.02E-10
Maximum =	0.4137918	0.4807454	1.553685	4.371781	2.326264	3.313776	12.46004
Mean =	0.1450174	0.1684819	0.544504	1.532133	0.8152617	1.161345	4.366743
Std Deviation =	0.1130973	0.1313971	0.4246524	1.194893	0.6358132	0.9057194	3.405572
Variance =	1.28E-02	1.73E-02	0.1803296	1.427768	0.4042584	0.8203276	11.59792
5% Perc =	3.08E-06	3.57E-06	1.15E-05	3.25E-05	1.73E-05	2.46E-05	9.26E-05
10% Perc =	5.82E-05	6.76E-05	2.19E-04	6.15E-04	3.27E-04	4.66E-04	1.75E-03
15% Perc =	5.19E-04	6.03E-04	1.95E-03	5.49E-03	2.92E-03	4.16E-03	1.56E-02
20% Perc =	3.46E-03	4.02E-03	1.30E-02	3.66E-02	1.95E-02	2.77E-02	0.1042169
25% Perc =	1.85E-02	2.14E-02	6.93E-02	0.1950503	0.103788	0.1478466	0.5559143
30% Perc =	0.0522569	6.07E-02	0.196492	0.5528917	0.2941987	0.4190877	1 573553
35% Perc =	8.42E-02	9.78E-02	0.3165513	0.8907163	0.4739582	0.6751561	2.536014
40% Perc =	0.1094766	0.1271905	0.4110574	1.156639	0.615458	0.8767233	3 296544
45% Perc =	0.1314802	0.1527544	0.4936756	1.389111	0.7391586	1.052935	3.959115
50% Perc =	0.151303	0.1757845	0.5681049	1.598541	0.8505983	1.211682	4.556014
55% Perc =	0 1680076	0.1951921	0.6308268	1.775029	0.944509	1 345458	5 059023
60% Perc =	0.1836091	0 2133179	0.6894398	1.939955	1.032268	1.470471	5.528811
65% Perc =	0.1987394	0 2308964	0.7462168	2.099715	1.117277	1.591568	5.984412
70% Perc =	0.2147695	0 2495202	0.806655	2.269777	1.207769	1.720473	6.467109
75% Perc =	0.2324509	0.2700626	0.8727952	2.455883	1.306798	1.86154	6.99953
80% Perc =	0 2517483	0.2924824	0.9452522	2.659763	1 415284	2.01608	7.580611
85% Perc =	0.2739266	0.3182493	1.028526	2.894081	1.539967	2.193691	8.248441
90% Perc =	0 2999293	0.3484593	1.12616	3.168803	1.686149	2.401929	9.031429
95% Perc =	0 3333622	0.3873019	1.251692	3.522028	1.874103	2.66967	10.03816

Table E17 - Brie risk assessment, contamination frequency (0, 0.3, 0.65) from Bemrah *et al* (1998)

rest of Australia	Susceptible population								Susceptible population
Name	Cancer	Transplant	AIDS	Diabetes	Pregant	Kidney	< 30 days	> 60 years	Total
Minimum =	4.80E-10	1.51E-09	1.20E-09	2.49E-09	3.21E-09	9.27E-11	3.50E-10	2.06E-09	1.14E-08
Maximum =	11.90356	37.56548	29.70021	61.79522	79.59187	2.300886	8.687017	51.20372	282.748
Mean =	4.171718	13.16519	10.40872	21.65673	27.89374	0.8063676	3 044449	17.94484	99.09176
Std Deviation =	3.253475	10.26738	8.117644	16.88983	21.754	0.6288768	2.374331	13.99497	77.28051
Variance =	10.5851	105.419	65.89615	285.2665	473 2366	0.395486	5.637446	195.8593	5972.277
5% Perc =	8.85E-05	2.79E-04	2 21E-04	4.59E-04	5 91E-04	1.71E-05	6 46E-05	3.81E-04	2.10E-03
10% Perc =	1.67E-03	5.28E-03	4.18E-03	8.69E-03	1.12E-02	3.24E-04	1.22E-03	7.20E-03	3.98E-02
15% Perc =	1.49E-02	4.71E-02	3.72E-02	7.77E-02	0.1000311	2.89E-03	1.09E-02	6.42E-02	0.3553579
20% Perc =	9.96E-02	0.3142009	0.2484151	0.5168605	0 6657131	0.0192448	7.27E-02	0.4282723	2.364928
25% Perc =	0.5310864	1.676012	1.325097	2.75704	3.551051	0.1026558	0.3875779	2.284493	12.61501
30% Perc =	1.503276	4.744065	3.750777	7.815137	10.06585	0.2909892	1.097065	6.466411	35.75866
35% Perc =	2.422753	7.645768	6.044935	12.59029	16 21622	0.4687876	1.768084	10.42158	57.6077
40% Perc =	3.149317	9.938671	7.857762	16.34912	21.05756	0.6087436	2.298318	13.54693	74.80642
45% Perc =	3.782296	11.93624	9.43709	19.63511	25.28991	0.7310947	2.760256	16.26972	89 84172
50% Perc =	4.352537	13.73582	10.85988	22.59542	29.10276	0.8413188	3.176408	18.72264	103.3868
55% Perc =	4.833081	15.25233	12.05887	25.09007	32.31586	0.9342049	3 5271	20.78972	114.8012
60% Perc =	5.281887	16.66868	13.17867	27.42131	35.31848	1.021006	3.854631	22 72028	125.4679
65% Perc =	5 717141	18.04226	14.26466	29.67952	38.22704	1.105088	4.172273	24.59255	135.8005
70% Perc =	6 17828	19.49753	15.41523	32.08334	41.32315	1.194593	4.508804	26.57616	146.7994
75% Perc =	6.686922	21.10271	16.68433	34.71396	44.71137	1.292541	4.880002	28.7641	158.8359
80% Perc =	7.242051	22.8546	18 06942	37.59581	48.42318	1.399844	5.285126	31.15202	172.022
85% Perc =	7.880054	24.86802	19 66128	40.9079	52.68912	1.523166	5.75073	33.89642	187.1767
90% Perc =	8.628075	27.22864	21.52764	44.79111	57.69067	1.667754	6.296622	37.11406	204.9446
95% Perc =	9.589841	30 2638	23.92731	49.78395	64.12142	1.853658	6.998503	41.25114	227.7896

Table E17 - Brie risk assessment, contamination frequency (0, 0.3, 0.65) from Bemrah *et al* (1998)

rest of Australia	General population						General population	Probability of
Name	1 - 9 years	10 - 19 years	20 - 29 years	30 - 39 years	40 - 49 years	50 - 59 years	Total	infection (per meal)
Minimum =	6.61E-11	7.68E-11	2.48E-10	6.98E-10	3.71E-10	5.29E-10	1.99E-09	7.55E-15
Maximum =	1.639532	1.904817	6.156036	17.32194	9.21716	13.1299	49.36938	2.31E-04
Mean =	0.57459	0.6675615	2.157442	6.070641	3.230243	4.601499	17.30198	7.19E-05
Std Deviation =	0.4481161	0.5206235	1.682564	4.734422	2.519229	3.588655	13.49361	5.95E-05
Variance =	0.200808	0.2710488	2.831022	22.41475	6.346514	12.87845	182.0775	3.55E-09
5% Perc =	1.22E-05	1.42E-05	4.57E-05	1 29E-04	6.85E-05	9.76E-05	3.67E-04	1.57E-09
10% Perc =	2 31E-04	2.68E-04	8.66E-04	2.44E-03	1.30E-03	1.85E-03	6.95E-03	3.03E-08
15% Perc =	2 05E-03	2.39E-03	7.72E-03	2.18E-02	1.16E-02	1.65E-02	6.19E-02	2.45E-07
20% Perc =	1.37E-02	1.59E-02	5.15E-02	0.1448822	7.71E-02	0 1098196	0.4129297	1.64E-06
25% Perc =	7.31E-02	8.50E-02	0.2746562	0.7728313	0.4112306	0 5858002	2.202652	9.75E-06
30% Perc =	0.2070531	0.2405553	0.7774328	2.190676	1.164016	1 658147	6.234755	2.74E-05
35% Perc =	0.3336969	0.3876907	1.252949	3.52921	1.875985	2.67235	10.04824	4.02E-05
40% Perc =	0.4337699	0.503956	1.628698	4.582852	2.438577	3.473767	13.06162	4.97E-05
45% Perc =	0.5209531	0.6052459	1.956049	5.503957	2.928705	4.171957	15.68687	5.77E-05
50% Perc =	0 599495	0.6964962	2 250954	6.333766	3.370254	4.800946	18.05191	6.59E-05
55% Perc =	0.6656825	0.7733932	2.499472	7.033048	3.742348	5.330996	20.04494	7.48E-05
60% Perc =	0.7274987	0.8452115	2.731576	7.68652	4.089868	5.826039	21.90634	8.39E-05
65% Perc =	0.7874482	0.9148612	2.956672	8.319523	4.426894	6.306134	23.71153	9.36E-05
70% Perc =	0.850963	0.988653	3.195154	8.993345	4 783962	6.814781	25.62408	1.04E-04
75% Perc =	0.9210206	1.070046	3.458203	9.730738	5.177814	7.375824	27.73365	1 15E-04
80% Perc =	0.9974811	1.158878	3.745293	10.53856	5 607661	7.988144	30.03601	1.27E-04
85% Perc =	1.085356	1.260972	4.075242	11.46697	6.10168	8.691876	32.6821	1.41E-04
90% Perc =	1.188385	1.380671	4.462088	12.55548	6.680886	9.516959	35.78447	1 59E-04
95% Perc =	1.320853	1.534574	4.959474	13 95504	7.425601	10.57781	39.77335	1.81E-04

Table E18 - Brie risk assessment, contamination frequency (0, 0, 0.003)

Tasmania Susceptible population — Susceptible population

Name	Cancer	Transplant	AIDS	Diabetes	Pregant	Kidney	< 30 days	> 60 years	Total
Minimum =	6.35E-13	2.00E-12	1 58E-12	3.30E-12	4.25E-12	1.23E-13	4.63E-13	2.73E-12	1.51E-11
Maximum =	0.0138407	4.37E-02	0.0345335	7.19E-02	9.25E-02	2.68E-03	1.01E-02	5.95E-02	0.328761213
Mean =	3 32E-03	1.05E-02	8.29E-03	0.0172404	2.22E-02	6.42E-04	2.42E-03	1.43E-02	4.08E-01
Std Deviation =	3.41E-03	1.08E-02	8.51E-03	1.77E-02	2.28E-02	6 59E-04	2.49E-03	1.47E-02	1.60E-01
Variance =	1.16E-05	1.16E-04	7.24E-05	3.14E-04	5.20E-04	4.35E-07	6.20E-06	2.15E-04	8 23E-02
Mode =	1.26E-10	3.99E-10	3.15E-10	6.56E-10	8.45E-10	2.44E-11	9.22E-11	5.44E-10	1.26E-03
5% Perc =	4 23E-08	1.33E-07	1.05E-07	2.19E-07	2.83E-07	8.17E-09	3.08E-08	1.82E-07	1.01E-06
10% Perc =	1.06E-06	3.34E-06	2.64E-06	5.50E-06	7.08E-06	2.05E-07	7.73E-07	4.56E-06	2.62E-05
15% Perc =	1 00E-05	3.16E-05	2.50E-05	5.20E-05	6.69E-05	1.93E-06	7.30E-06	4.31E-05	2.63E-04
20% Perc =	5.66E-05	1.79E-04	1.41E-04	2.94E-04	3.78E-04	1.09E-05	4.13E-05	2.43E-04	1.58E-03
25% Perc =	1 88E-04	5.92E-04	4.68E-04	9.74E-04	1.25E-03	3.63E-05	1.37E-04	8.07E-04	5.80E-03
30% Perc =	4.50E-04	1.42E-03	1.12E-03	2.34E-03	3.01E-03	8.70E-05	3.28E-04	1.94E-03	1.51E-02
35% Perc =	8.10E-04	2.56E-03	2.02E-03	4.20E-03	5.42E-03	1.57E-04	5.91E-04	3.48E-03	2.99E-02
40% Perc =	1.25E-03	3.94E-03	3.11E-03	6.48E-03	8.34E-03	2.41E-04	9.10E-04	5.37E-03	4.89E-02
45% Perc =	1.75E-03	5.51E-03	4.36E-03	9.06E-03	1.17E-02	3.38E-04	1.27E-03	7.51E-03	7.11E-02
50% Perc =	2.25E-03	7.11E-03	5.62E-03	1.17E-02	1.51E-02	4.35E-04	1.64E-03	9.69E-03	9.50E-02
55% Perc =	2.83E-03	8.92E-03	7.05E-03	1.47E-02	1.89E-02	5.46E-04	2.06E-03	1.22E-02	0.120662505
60% Perc =	3.43E-03	1.08E-02	8.55E-03	1.78E-02	2.29E-02	6.62E-04	2.50E-03	1.47E-02	1.49E-01
65% Perc =	4.08E-03	1.29E-02	1.02E-02	2.12E-02	2.73E-02	7.89E-04	2.98E-03	1.76E-02	1.78E-01
70% Perc =	4.82E-03	1.52E-02	1.20E-02	2.50E-02	3.22E-02	9.32E-04	3 52E-03	2.07E-02	2.11E-01
75% Perc =	5.59E-03	1.77E-02	0.0139589	2.90E-02	3.74E-02	1.08E-03	4.08E-03	2.41E-02	0.247415495
80% Perc =	6.46E-03	2.04E-02	1.61E-02	3.35E-02	4.32E-02	1 25E-03	4.71E-03	2.78E-02	0.286308955
85% Perc =	7.43E-03	2.35E-02	1.85E-02	0.038576	4.97E-02	1.44E-03	5.42E-03	0.0319642	0.329926196
90% Perc =	8.66E-03	2.73E-02	2.16E-02	4.49E-02	5.79E-02	1.67E-03	6.32E-03	3.72E-02	0.382169337
95% Perc =	1.02E-02	3.21E-02	2.54E-02	5.28E-02	6.80E-02	1.97E-03	7.42E-03	4.37E-02	0.447247694

Table E18 - Brie risk assessment, contamination frequency (0, 0, 0.003)

Tasmania General population

Name	1 - 9 years	10 - 19 years	20 - 29 years	30 - 39 years	40 - 49 years	50 - 59 years	General population Total
Minimum =	8.75E-14	1.02E-13	3.28E-13	9.24E-13	4 92E-13	7.00E-13	2.63E-12
Maximum =	1.91E-03	2.21E-03	7.16E-03	2.01E-02	1.07E-02	0.0152666	5.74E-02
Mean =	4.57E-04	5.31E-04	1.72E-03	4.83E-03	2.57E-03	3.66E-03	1.38E-02
Std Deviation =	4.70E-04	5 46E-04	1.76E-03	4.96E-03	2.64E-03	0 0037627	1.41E-02
Variance =	2.21E-07	2.98E-07	3.11E-06	2.46E-05	6.98E-06	1.42E-05	4.94E-05
Mode =	1.74E-11	2.02E-11	6.54E-11	1.84E-10	9.79E-11	2.68E-03	2.68E-03
5% Perc =	5.82E-09	6.76E-09	2.19E-08	6.15E-08	3.27E-08	4.66E-08	1.75E-07
10% Perc =	1.46E-07	1.70E-07	5.48E-07	1.54E-06	8.20E-07	1.17E-06	4.39E-06
15% Perc =	1.38E-06	1.60E-06	5.18E-06	1.46E-05	7.75E-06	1.11E-05	4.16E-05
20% Perc =	7.79E-06	9.05E-06	2.93E-05	8.23E-05	4.38E-05	6.24E-05	2.35E-04
25% Perc =	2.58E-05	3.00E-05	9.70E-05	2.73E-04	1.45E-04	2.07E-04	7.78E-04
30% Perc =	6.20E-05	7.20E-05	2.33E-04	6.55E-04	3.49E-04	4.96E-04	1.87E-03
35% Perc =	1.12E-04	1.30E-04	4.19E-04	1.18E-03	6.27E-04	8.94E-04	3.36E-03
40% Perc =	1.72E-04	2.00E-04	6.45E-04	1.82E-03	9.66E-04	1.38E-03	5.17E-03
45% Perc =	2 41E-04	2.79E-04	9.03E-04	2.54E-03	1.35E-03	1.93E-03	7.24E-03
50% Perc =	3.10E-04	3.60E-04	1.16E-03	3.28E-03	1.74E-03	2.48E-03	9.34E-03
55% Perc =	3.89E-04	4.52E-04	1.46E-03	4.11E-03	2.19E-03	3.12E-03	1.17E-02
60% Perc =	4 72E-04	5.48E-04	1.77E-03	4.99E-03	2.65E-03	3.78E-03	1.42E-02
65% Perc =	5.62E-04	6.53E-04	2.11E-03	5.94E-03	3.16E-03	4.50E-03	1.69E-02
70% Perc =	6.64E-04	7.72E-04	2.49E-03	7.02E-03	3.73E-03	5.32E-03	2.00E-02
75% Perc =	7.71E-04	8.95E-04	2.89E-03	8.14E-03	4.33E-03	6.17E-03	2.32E-02
80% Perc =	8.90E-04	1.03E-03	3.34E-03	9 40E-03	5.00E-03	7.12E-03	2 68E-02
85% Perc =	1.02E-03	1.19E-03	3.84E-03	1.08E-02	5.75E-03	8.20E-03	3.08E-02
90% Perc =	1.19E-03	1.39E-03	4.48E-03	1.26E-02	6.70E-03	9.55E-03	3.59E-02
95% Perc =	1.40E-03	1.63E-03	5.26E-03	0.0148002	7.88E-03	1.12E-02	4.22E-02

Table E18 - Brie risk assessment, contamination frequency (0, 0, 0.003)

rest of Australia	Susceptible population								Susceptible population
Name	Cancer	Transplant	AIDS	Diabetes	Pregant	Kidney	< 30 days	> 60 years	Total
Minimum =	2.52E-12	7.94E-12	6.28E-12	1.31E-11	1.68E-11	4.86E-13	1.84E-12	1.08E-11	5.98E-11
Maximum =	5.48E-02	0.1730646	0.1368292	0.2846912	0.3666805	0.0106002	4.00E-02	0.2358961	1.30E+00
Mean =	1.32E-02	4.15E-02	3.28E-02	6.83E-02	8.80E-02	2.54E-03	9.60E-03	5.66E-02	3 13E-01
Std Deviation =	1.35E-02	4.27E-02	3.37E-02	7.02E-02	9.04E-02	2.61E-03	9.86E-03	0.0581404	3.21E-01
Variance =	1.83E-04	1.82E-03	1.14E-03	4.92E-03	8.17E-03	6.83E-06	9 73E-05	3.38E-03	1.97E-02
Mode =	5.01E-10	1.58E-09	1.25E-09	2.60E-09	3.35E-09	9.68E-11	3.66E-10	2.15E-09	1.19E-08
5% Perc =	1.67E-07	5.29E-07	4.18E-07	8.69E-07	1.12E-06	3.24E-08	1.22E-07	7.20E-07	3.98E-06
10% Perc =	4.20E-06	1.32E-05	1.05E-05	2.18E-05	2.81E-05	8.11E-07	3.06E-06	1.81E-05	9.97E-05
15% Perc =	3.97E-05	1.25E-04	9.90E-05	2.06E-04	2.65E-04	7.67E-06	2.89E-05	1.71E-04	9.42E-04
20% Perc =	2.24E-04	7.07E-04	5.59E-04	1.16E-03	1.50E-03	4.33E-05	1.64E-04	9.64E-04	5.32E-03
25% Perc =	7.43E-04	2.35E-03	1.86E-03	3 86E-03	4.97E-03	1.44E-04	5.43E-04	3.20E-03	1.77E-02
30% Perc =	1.78E-03	5.63E-03	4.45E-03	9.26E-03	1.19E-02	3.45E-04	1.30E-03	7.67E-03	4.24E-02
35% Perc =	3.21E-03	1.01E-02	8.01E-03	0.0166607	2.15E-02	6.20E-04	2.34E-03	1.38E-02	7.62E-02
40% Perc =	4.94E-03	1.56E-02	0.0123317	2.57E-02	3.30E-02	9.55E-04	3.61E-03	2.13E-02	1.17E-01
45% Perc =	6.92E-03	2.18E-02	1.73E-02	3.59E-02	4.63E-02	1.34E-03	5.05E-03	0.0297606	1.64E-01
50% Perc =	8.93E-03	2.82E-02	2.23E-02	4.63E-02	5.97E-02	1.73E-03	6.51E-03	3.84E-02	2.12E-01
55% Perc =	1.12E-02	3.54E-02	2.80E-02	5.82E-02	0 074903	2.17E-03	8.18E-03	4.82E-02	2.66E-01
60% Perc =	1.36E-02	4.29E-02	3.39E-02	7.05E-02	9.08E-02	2.62E-03	9.91E-03	5.84E-02	3.23E-01
65% Perc =	1.62E-02	5.10E-02	4.04E-02	8.40E-02	0.108143	3.13E-03	1.18E-02	6.96E-02	3.84E-01
70% Perc =	1.91E-02	6.03E-02	4.77E-02	9.92E-02	0.1277352	3.69E-03	0 0139416	8.22E-02	4.54E-01
75% Perc =	0.022167	7.00E-02	5.53E-02	0.115076	0.1482172	4.28E-03	0.0161771	9.54E-02	5.27E-01
80% Perc =	2.56E-02	8.08E-02	6.39E-02	0.1328536	0.1711147	4.95E-03	1.87E-02	0.110083	6.08E-01
85% Perc =	2 94E-02	9.29E-02	7.35E-02	0.1528464	0.1968652	5.69E-03	2 15E-02	0.1266491	6 99E-01
90% Perc =	3 43E-02	0.1082636	8.56E-02	0.1780936	0.2293835	6.63E-03	2.50E-02	0.147569	8.15E-01
95% Perc =	4.03E-02	0.1271739	0.1005469	0.2092011	0.2694497	7.79E-03	2.94E-02	0.1733447	9.57E-01

Table E18 - Brie risk assessment, contamination frequency (0, 0, 0.003)

rest of Australia	General population						General population
Name	1 - 9 years	10 - 19 years	20 - 29 years	30 - 39 years	40 - 49 years	50 - 59 years	Total
Minimum =	3.47E-13	4.03E-13	1.30E-12	3.66E-12	1.95E-12	2.78E-12	1.04E-11
Maximum =	7.55E-03	8 78E-03	2.84E-02	7.98E-02	4.25E-02	6.05E-02	2.27E-01
Mean =	1.81E-03	2.11E-03	6.81E-03	1.91E-02	1.02E-02	1.45E-02	5.46E-02
Std Deviation =	1.86E-03	2.16E-03	6.99E-03	1.97E-02	1.05E-02	1.49E-02	5.61E-02
Variance =	3.47E-06	4 68E-06	4.89E-05	3.87E-04	1.10E-04	2.22E-04	7.76E-04
Mode =	6.90E-11	8.01E-11	2.59E-10	7.29E-10	3.88E-10	1.06E-02	1.06E-02
5% Perc =	2.31E-08	2.68E-08	8.66E-08	2.44E-07	1.30E-07	1.85E-07	6.95E-07
10% Perc =	5.78E-07	6.72E-07	2.17E-06	6.11E-06	3.25E-06	4.63E-06	1.74E-05
15% Perc =	5.46E-06	6.35E-06	2.05E-05	5.77E-05	3.07E-05	4.39E-05	1.65E-04
20% Perc =	3 09E-05	3.59E-05	1.16E-04	3.26E-04	1.74E-04	2.47E-04	9.30E-04
25% Perc =	1.02E-04	1 19E-04	3.85E-04	1.08E-03	5.76E-04	8.20E-04	3.08E-03
30% Perc =	2.46E-04	2.85E-04	9.22E-04	2.60E-03	1 38E-03	1.97E-03	7.40E-03
35% Perc =	4.42E-04	5.14E-04	1.66E-03	4.67E-03	2.49E-03	3.54E-03	1.33E-02
40% Perc =	6.81E-04	7.91E-04	2.56E-03	7.19E-03	3.83E-03	5.45E-03	2.05E-02
45% Perc =	9.53E-04	1.11E-03	3.58E-03	1.01E-02	5.36E-03	7.63E-03	2.87E-02
50% Perc =	1.23E-03	1.43E-03	4.62E-03	1.30E-02	6.91E-03	9.84E-03	3.70E-02
55% Perc =	1.54E-03	1.79E-03	5.79E-03	1.63E-02	8.67E-03	0.0123564	4.65E-02
60% Perc =	1.87E-03	2.17E-03	7.02E-03	1.98E-02	1.05E-02	0.0149855	5.63E-02
65% Perc =	2 23E-03	2.59E-03	8.36E-03	2.35E-02	1.25E-02	1.78E-02	6.71E-02
70% Perc =	2 63E-03	3.06E-03	9 88E-03	0.0277996	1.48E-02	2.11E-02	7.93E-02
75% Perc =	3 05E-03	3.55E-03	1.15E-02	3.23E-02	1.72E-02	2.45E-02	9.19E-02
80% Perc =	3 52E-03	4.10E-03	1.32E-02	3.72E-02	1.98E-02	2.82E-02	1.06E-01
85% Perc =	4.06E-03	4.71E-03	1.52E-02	4.28E-02	2.28E-02	3.25E-02	1.22E-01
90% Perc =	4.73E-03	5.49E-03	1.77E-02	4.99E-02	2.66E-02	3.78E-02	1.42E-01
95% Perc =	5.55E-03	6 45E-03	0.0208406	5.86E-02	3 12E-02	4.44E-02	1.67E-01

Appendix F – Product Case Study 2 – 1 kg Ricotta

Risk Assessment Results

Table F.1 - Bestfit function and goodness of fit statistics for Ricotta

Process Step	Function	Goodness of fit	
Ricotta Production		**Chi-Square**	**Kolmogorov-Smirnov**
Temperature	Normal	2.646572	0.114341
pH	Normal	10.193994	0.030301
Salt concentration	Normal	10.257344	0.072522
Final product specifications			
pH	Normal	9.581565	0.085338
Salt concentration	Normal	2.543782	0.07536
Storage and transport			
Temperature	Normal	7.544429	0.114341
Shelf life at 5°C			
pH	Normal	56.402182	0.089598
Salt concentration	Normal	0.508698	0.014504
Shelf life at 10°C			
pH	Normal	813.28868	0.052448
Salt concentration	Normal	1.603314	0.009027

Table F.2 - Input variable ranges

	Minimum	Mean	Maximum
Temperature	-3.9409	-0.000003	3.8766
pH	-3.7724	0.000003	3.7754
%NaCl	-3.7250	0.00002	3.7901

Table F.3 - Output values - Log growth for each stage of production process, and overall total amount of growth possible

	Minimum	Mean	Maximum	50th percentile	95th percentile
0 - 4 hr	0.2307	0.8740	1.0219	0.9070	1.0099
4 - 8 hr	0.0008	0.2201	1.0708	0.1769	0.5602
8 - 12 hr	0.0020	0.0488	0.3229	0.0408	0.1129
12 - 16 hr	0.0032	0.0412	0.2165	0.0365	0.0843
16 - 20 hr	0.0135	0.0493	0.1543	0.0469	0.0793
Total growth	0.2502	1.2334	2.2972	1.2199	1.8043

Table F.4 - Correlation of output values with Ricotta manufacture process parameter inputs values

	Temperature	pH	%NaCl
0 - 4 hr	0.8611	-0.2456	0.0068
4 - 8 hr	0.9996	0.0192	0.0041
8 - 12 hr	0.9968	0.0766	0.0049
12 - 16 hr	0.9952	0.0925	0.0070
16 - 20 hr	0.9898	0.1294	0.0182
Total growth	0.9963	-0.0400	0.0086

Table F.5 – Output values – Log predicted *L. monocytogenes* growth for storage and distribution stage

	Minimum	Mean	Maximum	50th percentile	95th percentile
0 - 4 hr	7.10e-3	4.65e-2	0.1984	4.30e-2	8.39e-2
4 - 8 hr	7.96e-3	5.04e-2	0.2114	4.69e-2	8.93e-2
8 - 12 hr	1.03e-2	4.83e-2	0.1727	4.57e-2	8.01e-2
12 - 16 hr	1.24e-2	4.47e-2	0.1356	4.29e-2	6.94e-2
16 - 20 hr	1.72e-2	4.29e-2	0.1043	4.18e-2	6.09e-2
20 –27 hrs	2.14e-2	7.39e-2	0.2550	7.02e-2	0.1183
Total growth	7.63e-2	0.3066	1.0776	0.2905	0.5020

Table F.6 – Correlation of output values with Ricotta storage and distribution stage parameter inputs values

	Temperature	pH	%NaCl
Total growth	0.9857	0.1423	0.0268

Table F.7 – Output values – Log predicted *L. monocytogenes* growth for each stage of shelf life

	Minimum	Mean	Maximum	50th percentile	95th percentile
Day 0 - 4	3.05	5.09	7.47	5.08	5.99
Day 4 - 8	1.79	3.28	5.22	3.26	3.95
Day 8 - 12	0.54	1.11	1.96	1.09	1.41
Day 12 - 16	0.24	0.54	1.01	0.53	0.71
Day 16 - 20	0.19	0.43	0.81	0.42	0.57
Day 20 –24	0.19	0.43	0.81	0.42	0.57
Total growth	6.21	11.31	18.10	11.24	13.65

Table F.8 – Correlation of output values with Ricotta shelf life inputs values

	Temperature	pH	%NaCl
Day 0 - 4	0.9962	0.0780	0.0376
Day 4 - 8	0.9193	0.3637	0.0331
Day 8 - 12	0.7535	0.6226	0.0283
Day 12 – 16	0.7139	0.6665	0.0255
Day 16 – 20	0.7339	0.6448	0.0247
Day 20 –24	0.7330	0.6459	0.0252
Total growth	0.9074	0.3893	0.0339

Table F9 - Ricotta detailed risk assessment outputs, shelf life storage at 5 °C

Name	Number of serves/annum	Susceptible population Cancer	Transplant	AIDS	Diabetes	Pregant	Kidney	> 60 years	< 30 days	Susceptible population Total	Probability of infection (per meal)
Minimum =	242026.7	3.42E-13	1.08E-12	8.53E-13	1.77E-12	2.29E-12	6 61E-14	2.49E-13	1.47E-12	8.12E-12	2.44E-15
Maximum =	5610906	4.35E-02	0.1373655	0.1086046	0.2259662	0.2910431	8.41E-03	3.18E-02	0.1869721	1.033658	2.22E-04
Mean =	759709.1	9.98E-04	3.15E-03	2.49E-03	5.18E-03	6.68E-03	1.93E-04	7 29E-04	4.29E-03	2.37E-02	5.28E-06
Std Deviation =	522004.3	4 11E-03	1.30E-02	1.02E-02	2.13E-02	2.74E-02	7.94E-04	3.00E-03	1.76E-02	9.75E-02	2.13E-05
Variance =	2.72E+11	1 69E-05	1.68E-04	1.05E-04	4.54E-04	7.53E-04	6.30E-07	8.98E-06	3.11E-04	9.50E-03	4.55E-10
Skewness =	2 67105	5 827673	5.827673	5.827673	5.827673	5.827673	5.827673	5 827673	5.827673	5.827673	5.699864
5% Perc =	296648	2 63E-10	8.30E-10	6.56E-10	1.37E-09	1.76E-09	5.08E-11	1.92E-10	1.13E-09	6.25E-09	1.35E-12
10% Perc =	328797.4	1.06E-09	3 35E-09	2.65E-09	5.51E-09	7.09E-09	2.05E-10	7.74E-10	4.56E-09	2.52E-08	5.63E-12
15% Perc =	358593.8	2 84E-09	8.95E-09	7.07E-09	1.47E-08	1.90E-08	5.48E-10	2 07E-09	1.22E-08	6.73E-08	1 63E-11
20% Perc =	388312.4	6.90E-09	2.18E-08	1.72E-08	3.58E-08	4.61E-08	1.33E-09	5.04E-09	2.96E-08	1.64E-07	3.69E-11
25% Perc =	418848.8	1.46E-08	4 62E-08	3.65E-08	7.60E-08	9.78E-08	2 83E-09	1.07E-08	6.29E-08	3.47E-07	7.88E-11
30% Perc =	450996.4	3.00E-08	9 46E-08	7.48E-08	1.56E-07	2.01E-07	5.80E-09	2.19E-08	1.29E-07	7.12E-07	1.57E-10
35% Perc =	485161	5.85E-08	1.85E-07	1.46E-07	3.04E-07	3.91E-07	1.13E-08	4.27E-08	2.51E-07	1.39E-06	3.12E-10
40% Perc =	521930.7	1.18E-07	3.73E-07	2.95E-07	6.14E-07	7.91E-07	2.29E-08	8 63E-08	5.08E-07	2.81E-06	6.10E-10
45% Perc =	561986.2	2.42E-07	7.64E-07	6.04E-07	1 26E-06	1.62E-06	4.68E-08	1.77E-07	1.04E-06	5.75E-06	1.24E-09
50% Perc =	606001 6	4.77E-07	1.51E-06	1.19E-06	2.48E-06	3.19E-06	9.22E-08	3.48E-07	2.05E-06	1.13E-05	2.59E-09
55% Perc =	654710	1.02E-06	3.21E-06	2.54E-06	5 28E-06	6 80E-06	1 96E-07	7.42E-07	4.37E-06	2.41E-05	5.55E-09
60% Perc =	709254.1	2.27E-06	7.18E-06	5.67E-06	1.18E-05	1.52E-05	4.39E-07	1.66E-06	9.77E-06	5.40E-05	1.20E-08
65% Perc =	770817 1	5.45E-06	1.72E-05	1.36E-05	2.83E-05	3.64E-05	1.05E-06	3.98E-06	2.34E-05	1.29E-04	2.91E-08
70% Perc =	841024.7	1.37E-05	4.31E-05	3.41E-05	7.09E-05	9.13E-05	2 64E-06	9.96E-06	5.86E-05	3.24E-04	6.99E-08
75% Perc =	922043.1	3.81E-05	1.20E-04	9.51E-05	1 98E-04	2.55E-04	7.37E-06	2.78E-05	1.64E-04	9.05E-04	1.90E-07
80% Perc =	1016978	9.98E-05	3.15E-04	2.49E-04	5 18E-04	6 67E-04	1.93E-05	7.28E-05	4.29E-04	2.37E-03	5.26E-07
85% Perc =	1144454	3.15E-04	9.94E-04	7.86E-04	1.64E-03	2.11E-03	6.09E-05	2.30E-04	1.35E-03	7.48E-03	1.74E-06
90% Perc =	1343603	1.13E-03	3.55E-03	2.81E-03	5.84E-03	7.53E-03	2.18E-04	8.21E-04	4 83E-03	0.0267258	6.69E-06
95% Perc =	1738756	5.67E-03	1.79E-02	1.41E-02	2.94E-02	3.79E-02	1.10E-03	4.14E-03	2.43E-02	0 134576	3.12E-05

Table F9 - Ricotta detailed risk assessment outputs, shelf life storage at 5 °C

Name	Level at end of storage	Level at consumption	General population 1 - 9 years	10 - 19 years	20 - 29 years	30 - 39 years	40 - 49 years	50 - 59 years	General population Total	Total from Buchanan R-values
Minimum =	-2.967387	2.85E-03	4.70E-14	5.46E-14	1.77E-13	4.97E-13	2.64E-13	3.77E-13	1.42E-12	6.01E-08
Maximum =	3.928465	1.00E+08	0.0059892	6.96E-03	0.0224834	6.33E-02	3.37E-02	4.80E-02	0 1803113	6395.7428
Mean =	-0.2785696	5244396	1.37E-04	1.60E-04	5.16E-04	1.45E-03	7.72E-04	1.10E-03	4.14E-03	142.59286
Std Deviation =	1.259955	1.88E+07	5.65E-04	6.56E-04	2.12E-03	5.97E-03	3.17E-03	4.52E-03	1.70E-02	560.9716
Variance =	1.587487	3.54E+14	3.19E-07	4.30E-07	4.50E-06	3.56E-05	1.01E-05	2.05E-05	2.89E-04	235092 24
Skewness =	0.3193123	4.254723	5.827673	5.827673	5 827673	5 827673	5.827673	5.827673	5.827673	11.382296
5% Perc =	-2 193356	1.661618	3.62E-11	4.20E-11	1.36E-10	3.82E-10	2.03E-10	2.90E-10	1.09E-09	4.63E-05
10% Perc =	-1.864329	6.821985	1.46E-10	1.69E-10	5.48E-10	1.54E-09	8.20E-10	1.17E-09	4.39E-09	1.87E-04
15% Perc =	-1.613462	18.43956	3.90E-10	4.53E-10	1.46E-09	4.12E-09	2.19E-09	3.12E-09	1.17E-08	4.99E-04
20% Perc =	-1.401327	44.23988	9 50E-10	1.10E-09	3.56E-09	1.00E-08	5.34E-09	7 61E-09	2.86E-08	1.21E-03
25% Perc =	-1.221497	89.67437	2.01E-09	2.34E-09	7.56E-09	2 13E-08	1.13E-08	1.61E-08	6.06E-08	2.57E-03
30% Perc =	-1.051312	178.9305	4.13E-09	4.79E-09	1.55E-08	4.36E-08	2.32E-08	3.31E-08	1.24E-07	5.27E-03
35% Perc =	-0.9004455	358.7843	8.05E-09	9.35E-09	3.02E-08	8.50E-08	4.52E-08	6.45E-08	2.42E-07	1.03E-02
40% Perc =	-0.7390609	735.9258	1.63E-08	1.89E-08	6.11E-08	1.72E-07	9.15E-08	1.30E-07	4.90E-07	2.08E-02
45% Perc =	-0.5748221	1422.892	3.33E-08	3.87E-08	1.25E-07	3.52E-07	1.87E-07	2.67E-07	1.00E-06	4.26E-02
50% Perc =	-0.409338	2929.03	6.56E-08	7.62E-08	2.46E-07	6.93E-07	3.69E-07	5.26E-07	1.98E-06	8.39E-02
55% Perc =	-0.2300974	6090.703	1.40E-07	1.62E-07	5.25E-07	1.48E-06	7.86E-07	1.12E-06	4.21E-06	0.17870467
60% Perc =	-4.19E-02	13463 89	3.13E-07	3.63E-07	1.17E-06	3.30E-06	1.76E-06	2.51E-06	9.42E-06	0.39977372
65% Perc =	0.1576615	31860.97	7.50E-07	8.70E-07	2.81E-06	7.92E-06	4.21E-06	6.00E-06	2.26E-05	0.9577907
70% Perc =	0.3747626	83102.41	1.88E-06	2.18E-06	7.05E-06	1.98E-05	1.06E-05	1.50E-05	5.66E-05	2.4003944
75% Perc =	0.6037753	221518 7	5.25E-06	6.09E-06	1.97E-05	5.54E-05	2.95E-05	4.20E-05	1.58E-04	6 6947882
80% Perc =	0.8617792	603462.5	1.37E-05	1.60E-05	5 16E-05	1.45E-04	7.72E-05	1.10E-04	4.14E-04	17.53413
85% Perc =	1.162632	1933339	4.33E-05	5.03E-05	1.63E-04	4.58E-04	2.44E-04	3.47E-04	1.30E-03	55.229213
90% Perc =	1.515601	7287636	1.55E-04	1.80E-04	5.81E-04	1.64E-03	8.70E-04	1.24E-03	4.66E-03	190 5287
95% Perc =	1.968189	3.55E+07	7.80E-04	9.06E-04	2.93E-03	8.23E-03	4.38E-03	6.25E-03	2.35E-02	876 4238

Table F10 - Ricotta risk assessment outputs, shelf life storage at 10 °C

	Susceptible population								Susceptible population	Probability of
Name	Cancer	Transplant	AIDS	Diabetes	Pregant	Kidney	< 30 days	> 60 years	Total	infection (per meal)
Minimum =	2.43E-12	7.66E-12	6.06E-12	1.26E-11	1.62E-11	4.69E-13	1.77E-12	1.04E-11	5.76E-11	6.66E-15
Maximum =	4.65E-02	0.1468514	0.1161044	0.2415706	0.3111415	8.99E-03	0.0339594	0.1998837	1.105039	2.32E-04
Mean =	1.22E-02	3.85E-02	0.0304352	6.33E-02	8.16E-02	2.36E-03	8.90E-03	5.24E-02	0.2896711	6.32E-05
Std Deviation =	1.16E-02	3.66E-02	2.90E-02	6.03E-02	7.77E-02	2.24E-03	8.48E-03	0.0498849	0.2757842	5.84E-05
Variance =	1.35E-04	1.34E-03	8.40E-04	3.63E-03	6.03E-03	5.04E-06	7.18E-05	2.49E-03	7.61E-02	3.41E-09
Skewness =	0.7380198	0.7380198	0.7380198	0.7380198	0.7380198	0.7380198	0.7380198	0.7380198	0.7380198	0.6631027
5% Perc =	1.24E-07	3.91E-07	3.09E-07	6.44E-07	8.29E-07	2.40E-08	9.05E-08	5.33E-07	2.94E-06	6.02E-10
10% Perc =	4.14E-06	1.31E-05	1.03E-05	2.15E-05	2.77E-05	8.01E-07	3.02E-06	1.78E-05	9.84E-05	2.28E-08
15% Perc =	3.97E-05	1.25E-04	9.89E-05	2.06E-04	2.65E-04	7.67E-06	2.89E-05	1.70E-04	9.41E-04	2.10E-07
20% Perc =	2.05E-04	6.47E-04	5.11E-04	1.06E-03	1.37E-03	3.96E-05	1.50E-04	8.80E-04	4.87E-03	1.19E-06
25% Perc =	8.02E-04	2.53E-03	2.00E-03	4.16E-03	5.36E-03	1.55E-04	5.85E-04	3.44E-03	1.90E-02	4.20E-06
30% Perc =	2.01E-03	6.35E-03	5.02E-03	1.04E-02	1.34E-02	3.89E-04	1.47E-03	8.63E-03	4.77E-02	1.17E-05
35% Perc =	3.87E-03	0.0122218	9.66E-03	2.01E-02	2.59E-02	7.49E-04	2.83E-03	1.66E-02	9.19E-02	2.35E-05
40% Perc =	6.01E-03	1.90E-02	1.50E-02	3.12E-02	4.02E-02	1.16E-03	4.38E-03	2.58E-02	0.1426145	3.49E-05
45% Perc =	8.07E-03	2.55E-02	2.01E-02	4.19E-02	5.40E-02	1.56E-03	5.89E-03	3.47E-02	0.1916686	4.52E-05
50% Perc =	9.97E-03	0.0314506	2.49E-02	5.17E-02	6.66E-02	1.93E-03	7.27E-03	4.28E-02	0.236662	5.38E-05
55% Perc =	1.17E-02	3.71E-02	2.93E-02	6.10E-02	7.85E-02	2.27E-03	8.57E-03	5.04E-02	0.2788303	6.18E-05
60% Perc =	1.36E-02	4.28E-02	0.0338721	7.05E-02	9.08E-02	2.62E-03	9.91E-03	5.83E-02	0.3223273	7.09E-05
65% Perc =	1.55E-02	4.89E-02	3.87E-02	8.05E-02	0.103638	3.00E-03	1.13E-02	6.66E-02	0.368077	8.06E-05
70% Perc =	1.77E-02	5.58E-02	4.41E-02	9.18E-02	0.118221	3.42E-03	1.29E-02	7.59E-02	0.4198697	9.13E-05
75% Perc =	0.0201348	6.35E-02	5.02E-02	0.1045262	0.1346292	3.89E-03	1.47E-02	8.65E-02	0.4781442	1.04E-04
80% Perc =	2.29E-02	7.22E-02	5.71E-02	0.1187965	0.1530092	4.42E-03	1.67E-02	9.83E-02	0.543422	1.17E-04
85% Perc =	2.60E-02	0.0820564	6.49E-02	0.1349828	0.173857	5.03E-03	1.90E-02	0.1116893	0.6174644	1.33E-04
90% Perc =	2.97E-02	9.39E-02	7.42E-02	0.1544011	0.1988677	5.75E-03	2.17E-02	0.1277567	0.7062916	1.51E-04
95% Perc =	3.49E-02	0.110109	8.71E-02	0.1811293	0.2332934	6.74E-03	0.0254627	0.1498725	0.8285567	1.75E-04

Table F10 - Ricotta risk assessment outputs, shelf life storage at 10 °C

	Level at	Level at time	General population						General population
Name	end of storage	of consumption	1 - 9 years	10 - 19 years	20 - 29 years	30 - 39 years	40 - 49 years	50 - 59 years	Total
Minimum =	-2.97278	1.20E-02	3.34E-13	3.88E-13	1.25E-12	3.53E-12	1.88E-12	2.67E-12	1.01E-11
Maximum =	4.119998	1.00E+08	6.40E-03	7.44E-03	2.40E-02	6.76E-02	3.60E-02	5.13E-02	0.192763
Mean =	-0.279081	6.33E+07	1.68E-03	1.95E-03	6.30E-03	1.77E-02	9.43E-03	1.34E-02	5.05E-02
Std Deviation =	1.26111	4.48E+07	1.60E-03	1.86E-03	6.00E-03	1.69E-02	8.98E-03	1.28E-02	4.81E-02
Variance =	1.590398	2.00E+15	2.55E-06	3.44E-06	3.60E-05	2.85E-04	8.07E-05	1.64E-04	2.31E-03
Skewness =	0.3055881	-0.5055441	0.7380198	0.7380198	0.7380198	0.7380198	0.7380198	0.7380198	0.7380198
5% Perc =	-2.198627	745.3793	1.71E-08	1.98E-08	6.40E-08	1.80E-07	9.59E-08	1.37E-07	5.14E-07
10% Perc =	-1.86825	26799.35	5.70E-07	6.62E-07	2.14E-06	6.02E-06	3.20E-06	4.56E-06	1.72E-05
15% Perc =	-1 612028	248430.8	5.46E-06	6.33E-06	2.05E-05	5.76E-05	3.06E-05	4.37E-05	1.64E-04
20% Perc =	-1.407483	1332354	2.82E-05	3.27E-05	1.06E-04	2.98E-04	1.58E-04	2.26E-04	8.49E-04
25% Perc =	-1.226712	5143330	1.10E-04	0.0001281	4.14E-04	1.16E-03	6.20E-04	8.83E-04	3.32E-03
30% Perc =	-1.054262	1.42E+07	2.77E-04	3.21E-04	1.04E-03	2.92E-03	1.55E-03	2.22E-03	8.34E-03
35% Perc =	-0.9027835	3.11E+07	5.33E-04	6.18E-04	2 00E-03	5.63E-03	2.99E-03	4.27E-03	1.60E-02
40% Perc =	-0.743533	6.39E+07	8.26E-04	9.60E-04	3.10E-03	8.73E-03	4.64E-03	6.62E-03	2.49E-02
45% Perc =	-0 5741563	1 00E+08	1.11E-03	1.29E-03	4.17E-03	1.17E-02	6.24E-03	8.89E-03	3.34E-02
50% Perc =	-0 4046941	1.00E+08	1.37E-03	1.59E-03	5.15E-03	1.45E-02	7.71E-03	1.10E-02	0 0412833
55% Perc =	-0.226479	1.00E+08	1.62E-03	1.88E-03	6.06E-03	1.71E-02	9.08E-03	1.29E-02	4 86E-02
60% Perc =	-3.18E-02	1.00E+08	1.87E-03	2.17E-03	7.01E-03	1.97E-02	0.0104971	0.0149609	5.62E-02
65% Perc =	0.1705377	1.00E+08	2.13E-03	2.48E-03	8.01E-03	2 25E-02	1.20E-02	1.71E-02	6.42E-02
70% Perc =	0.3777223	1.00E+08	2.43E-03	2.83E-03	9.14E-03	2 57E-02	1.37E-02	1 95E-02	7.33E-02
75% Perc =	0 6155809	1.00E+08	2.77E-03	3 22E-03	1.04E-02	2 93E-02	1.56E-02	0.0221894	8.34E-02
80% Perc =	0.8710011	1.00E+08	3.15E-03	3.66E-03	1.18E-02	3.33E-02	0.0176974	2.52E-02	9.48E-02
85% Perc =	1.165984	1.00E+08	3.58E-03	4 15E-03	1.34E-02	0.0377837	2.01E-02	2 87E-02	0.1077105
90% Perc =	1.504022	1.00E+08	4.09E-03	4.75E-03	1.54E-02	4.32E-02	2.30E-02	3.28E-02	0.1232055
95% Perc =	1.963057	1.00E+08	4.80E-03	5.58E-03	1.80E-02	0.0507008	2.70E-02	3.85E-02	0.1445334

Table F11 - Ricotta risk assessment, 21 day shelf life

Name	Level at end of storage	Level at time of consumption	Probability of infection (per meal)	Susceptible population Total	General population Total
Minimum =	-2.970824	2.90E-03	0.00E+00	0	0
Maximum =	4.015582	1.00E+08	1.37E-04	0.6772855	0.1181457
Mean =	-0.2791333	142049	1 45E-07	6.55E-04	1.14E-04
Std Deviation =	1.26219	1701602	2.11E-06	9.14E-03	1.59E-03
Variance =	1 593122	2.90E+12	4.46E-12	8.36E-05	2.54E-06
Skewness =	0.3131802	37.21434	42.91502	49.96992	49.96992
5% Perc =	-2.188776	0.7896281	6.46E-13	2.92E-09	5.09E-10
10% Perc =	-1.870927	2.8298	2.37E-12	1.04E-08	1 81E-09
15% Perc =	-1.624883	6.890796	5.73E-12	2.58E-08	4.51E-09
20% Perc =	-1.405816	14.12964	1.25E-11	5.20E-08	9.07E-09
25% Perc =	-1.217459	26.21513	2.35E-11	1.02E-07	1.78E-08
30% Perc =	-1.058923	47 55079	4 08E-11	1.82E-07	3.18E-08
35% Perc =	-0.9016367	81.60928	6.98E-11	3.09E-07	5.38E-08
40% Perc =	-0.7443244	140.7629	1.20E-10	5.43E-07	9.47E-08
45% Perc =	-0 578338	225.8671	2.04E-10	8.92E-07	1.56E-07
50% Perc =	-0.4078525	373.8991	3.31E-10	1.47E-06	2.57E-07
55% Perc =	-0.2293889	628.5089	5.66E-10	2.41E-06	4 20E-07
60% Perc =	-0.0463306	1073.764	9.79E-10	4.22E-06	7.36E-07
65% Perc =	0.1572056	1885.816	1.67E-09	7.47E-06	1.30E-06
70% Perc =	0.3797809	3436.323	3.13E-09	1.32E-05	2.30E-06
75% Perc =	0.613534	6621 686	5.93E-09	2.58E-05	4.50E-06
80% Perc =	0.8710024	13690.44	1.24E-08	5.20E-05	9.07E-06
85% Perc =	1.168648	30609.73	2.77E-08	1.22E-04	2.13E-05
90% Perc =	1.513408	80801 7	7.50E-08	3.14E-04	5.47E-05
95% Perc =	1.984305	310140.3	2.88E-07	1.34E-03	2.33E-04

Appendix G – Product Case Study 3 – 1 kg Mascarpone Risk Assessment Results

Table G.1 - Bestfit function and goodness of fit statistics for Mascarpone

Process Step	Function	Goodness of fit	
Mascarpone Production		**Chi-Square**	**Kolmogorov-Smirnov**
Temperature	Normal	4.242171	0.076209
pH	Normal	59.618878	0.148269
Salt concentration	Normal	5.031875	0.111388
Final product specifications			
pH	Normal	15.725761	0.043436
Salt concentration	Normal	2.914712	0.119788
Shelf life			
pH	Normal	5.912617	0.010775
Salt concentration	Normal	40.345611	0.118618

Table G.2 - Input variable ranges

	Minimum	Mean	Maximum
Temperature	-4.0454	-0.00002	4.0017
pH	-3.7666	0.00002	3.9240
%NaCl	-3.7700	-0.0000003	3.9186

Table G.3 – Output values – Predicted *L. monocytogenes* growth got Mascarpone production stage

	Minimum	Mean	Maximum	50th percentile	95th percentile
0 - 4 hr	5.93e-3	0.6148	0.7757	0.6557	0.7644
4 - 8 hr	5.38e-3	9.90e-2	0.4562	9.22e-2	0.1807
8 - 12 hr	1.13e-2	0.6562	0.8483	0.6961	0.8285
12 - 17 hr	1.31e-2	0.1415	0.5526	0.1337	0.2413
Total growth	2.43e-2	0.7978	1.3326	0.8307	1.0662

Table G.4 - Correlation of output values with input values

	Temperature	pH	%NaCl
0 - 4 hr	0.899	0.301	-0.023
4 - 8 hr	0.954	0.254	0.018
8 - 12 hr	0.852	0.467	0.033
12 - 17 hr	0.731	0.629	0.041
Total growth	0.921	0.304	-0.002

Table G5 - Mascarpone risk assessment, shelf life storage at 5 °C

	Level at	Level at time		General population						General population
Name	end of storage	of consumption	Dose	1 - 9 years	10 - 19 years	20 - 29 years	30 - 39 years	40 - 49 years	50 - 59 years	Total
Minimum =	-2.942039	1.62E-03	2.83E-02	0	0	0	0	0	0	0
Maximum =	3.270647	1.00E+08	1.15E+10	1.68E-03	1.96E-03	6.32E-03	0.0177955	9.47E-03	1.35E-02	5.07E-02
Mean =	-0.3175548	2450507	1.26E+08	1.74E-05	2.02E-05	6.52E-05	1.84E-04	9.78E-05	1.39E-04	5.24E-04
Std Deviation =	1.24925	1.37E+07	7.69E+08	1.11E-04	1.29E-04	4.18E-04	1.18E-03	6.26E-04	8.92E-04	3.35E-03
Variance =	1.560626	1.88E+14	5.91E+17	1.24E-08	1.67E-08	1.75E-07	1.38E-06	3.92E-07	7.96E-07	1.12E-05
Skewness =	0.3035854	6.460947	8.331558	8.846871	8.846871	8.846871	8.846871	8.846871	8.846871	8.846871
5% Perc =	-2.216531	0.401442	16.69716	2.17E-12	2.52E-12	8.13E-12	2.29E-11	1.22E-11	1.74E-11	6.52E-11
10% Perc =	-1.893528	1.298617	58.00359	7.54E-12	8.76E-12	2.83E-11	7.97E-11	4.24E-11	6.04E-11	2.27E-10
15% Perc =	-1.644956	2.833571	125.7956	1.66E-11	1.93E-11	6.22E-11	1.75E-10	9.33E-11	1.33E-10	4.99E-10
20% Perc =	-1.435954	5.430049	246.4079	3.20E-11	3.71E-11	1.20E-10	3.38E-10	1.80E-10	2.56E-10	9.63E-10
25% Perc =	-1.253863	9.28109	414.0946	5.71E-11	6.64E-11	2.14E-10	6.04E-10	3.21E-10	4.58E-10	1.72E-09
30% Perc =	-1.086037	15.55606	727.9273	9.47E-11	1.10E-10	3.56E-10	1.00E-09	5.33E-10	7.59E-10	2.85E-09
35% Perc =	-0.9349147	25.46154	1201.631	1.60E-10	1.86E-10	6.01E-10	1.69E-09	9.00E-10	1.28E-09	4.82E-09
40% Perc =	-0.7805411	43.79089	2054.69	2.69E-10	3.12E-10	1.01E-09	2.84E-09	1.51E-09	2.15E-09	8.10E-09
45% Perc =	-0.6141849	74.01891	3478.5	4.43E-10	5.15E-10	1.66E-09	4.69E-09	2.49E-09	3.55E-09	1.34E-08
50% Perc =	-0.4492555	129.3472	6183.32	8.08E-10	9.39E-10	3.03E-09	8.54E-09	4.55E-09	6.47E-09	2.43E-08
55% Perc =	-0.2699505	238.5995	11670.74	1.53E-09	1.78E-09	5.75E-09	1.62E-08	8.63E-09	1.23E-08	4.62E-08
60% Perc =	-8.14E-02	477.4029	22468.79	3.00E-09	3.48E-09	1.12E-08	3.17E-08	1.69E-08	2.40E-08	9.03E-08
65% Perc =	0.1193277	1029.594	49189.31	6.79E-09	7.89E-09	2.55E-08	7.18E-08	3.82E-08	5.44E-08	2.05E-07
70% Perc =	0.3346494	2617.193	124495.3	1.67E-08	1.94E-08	6.26E-08	1.76E-07	9.38E-08	1.34E-07	5.02E-07
75% Perc =	0.5698349	6756.18	314308.8	4.27E-08	4.96E-08	1.60E-07	4.52E-07	2.40E-07	3.42E-07	1.29E-06
80% Perc =	0.8263234	19838.64	955650.1	1.17E-07	1.36E-07	4.39E-07	1.24E-06	6.58E-07	9.37E-07	3.52E-06
85% Perc =	1.117712	71679.09	3556688	4.17E-07	4.85E-07	1.57E-06	4.41E-06	2.35E-06	3.34E-06	1.26E-05
90% Perc =	1.469206	341822.2	1.64E+07	2.17E-06	2.53E-06	8.16E-06	2.30E-05	1.22E-05	1.74E-05	6.55E-05
95% Perc =	1.921704	3826676	1.85E+08	2.52E-05	2.93E-05	9.45E-05	2.66E-04	1.42E-04	2.02E-04	7.59E-04

Table G5 - Mascarpone risk assessment, shelf life storage at 5 °C

Name	Number of serves/annum	Susceptible population Cancer	Transplant	AIDS	Diabetes	Pregant	Kidney	< 30 days	> 60 years	Susceptible population Total	Probability of infection (per meal)
Minimum =	13014	0	0	0	0	0	0	0	0	0	0
Maximum =	298112	1.22E-02	0.0385979	3.05E-02	6.35E-02	0.0817793	2.36E-03	8.93E-03	5.26E-02	0.2904998	2.14E-04
Mean =	40942	1 26E-04	3.99E-04	3.15E-04	6.56E-04	8.44E-04	2.44E-05	9.22E-05	5.43E-04	3.00E-03	2.35E-06
Std Deviation =	28134	8.09E-04	2.55E-03	2.02E-03	4.20E-03	5.41E-03	1.56E-04	5.90E-04	3.48E-03	1 92E-02	1.44E-05
Variance =	5 54E+09	6.54E-07	6.52E-06	4.07E-06	1.76E-05	2.92E-05	2.44E-08	3 48E-07	1.21E-05	3.69E-04	2.07E-10
Skewness =	3	8 846871	8 846871	8.846871	8.846871	8.846871	8.846871	8.846871	8.846871	8 846871	8 331285
5% Perc =	15987	1.57E-11	4.97E-11	3.93E-11	8 17E-11	1.05E-10	3.04E-12	1.15E-11	6.77E-11	3.74E-10	3.12E-13
10% Perc =	17718	5.48E-11	1.73E-10	1 37E-10	2.84E-10	3 66E-10	1.06E-11	4.00E-11	2.35E-10	1.30E-09	1.08E-12
15% Perc =	19328	1.20E-10	3.80E-10	3.01E-10	6.25E-10	8 05E-10	2.33E-11	8.79E-11	5.18E-10	2.86E-09	2.35E-12
20% Perc =	20926	2.32E-10	7.33E-10	5.79E-10	1.21E-09	1.55E-09	4.49E-11	1.69E-10	9.98E-10	5.51E-09	4.61E-12
25% Perc =	22573	4 15E-10	1.31E-09	1.04E-09	2.15E-09	2.77E-09	8.02E-11	3.03E-10	1.78E-09	9.86E-09	7.74E-12
30% Perc =	24305	6.88E-10	2.17E-09	1.72E-09	3.57E-09	4.60E-09	1.33E-10	5.02E-10	2.96E-09	1.63E-08	1.36E-11
35% Perc =	26145	1.16E-09	3.67E-09	2.90E-09	6.04E-09	7.77E-09	2.25E-10	8.48E-10	5.00E-09	2.76E-08	2.26E-11
40% Perc =	28129	1.95E-09	6.16E-09	4.87E-09	1.01E-08	1.31E-08	3.78E-10	1.43E-09	8.40E-09	4.64E-08	3.84E-11
45% Perc =	30288	3.22E-09	1.02E-08	8.04E-09	1.67E-08	2.15E-08	6.23E-10	2.35E-09	1.39E-08	7 65E-08	6.50E-11
50% Perc =	32661	5.87E-09	1.85E-08	1.46E-08	3.05E-08	3.92E-08	1.13E-09	4.28E-09	2.52E-08	1 39E-07	1.16E-10
55% Perc =	35287	1.11E-08	3.52E-08	2.78E-08	5 78E-08	7.45E-08	2.15E-09	8.13E-09	4.79E-08	2.65E-07	2.18E-10
60% Perc =	38222	2.18E-08	6.87E-08	5.43E-08	1.13E-07	1.46E-07	4.21E-09	1 59E-08	9.36E-08	5.17E-07	4.21E-10
65% Perc =	41535	4.94E-08	1.56E-07	1 23E-07	2.56E-07	3.30E-07	9.54E-09	3 60E-08	2.12E-07	1.17E-06	9.20E-10
70% Perc =	45326	1.21E-07	3.82E-07	3.02E-07	6.29E-07	8.10E-07	2.34E-08	8 84E-08	5 21E-07	2.88E-06	2.33E-09
75% Perc =	49691	3 10E-07	9.79E-07	7.74E-07	1.61E-06	2.07E-06	6.00E-08	2.26E-07	1 33E-06	7.37E-06	5.88E-09
80% Perc =	54814	8.50E-07	2.68E-06	2.12E-06	4.41E-06	5 68E-06	1.64E-07	6.20E-07	3.65E-06	2.02E-05	1.79E-08
85% Perc =	61660	3.03E-06	9.57E-06	7.57E-06	1.57E-05	2.03E-05	5.86E-07	2.21E-06	1.30E-05	7.20E-05	6.65E-08
90% Perc =	72410	1.58E-05	4.99E-05	3.94E-05	8.20E-05	1.06E-04	3.05E-06	1.15E-05	6 79E-05	3.75E-04	3.06E-07
95% Perc =	93727	1.83E-04	5.77E-04	4.57E-04	9.50E-04	1.22E-03	3.54E-05	1.34E-04	7.87E-04	0.0043464	3.45E-06

Table G6- Mascarpone risk assessment, shelf life storage at 10 °C

Name	Level at end of storage	Level at time of consumption	General populat 1 - 9 years	10 - 19 years	20 - 29 years	30 - 39 years	40 - 49 years	50 - 59 years	General population Total
Minimum =	-2 967924	5.13E-03	3.18E-13	3 70E-13	1.19E-12	3.36E-12	1.79E-12	2.55E-12	1.95E-11
Maximum =	2.954882	1.00E+08	1.22E-02	1.42E-02	4.59E-02	0.1293327	6.88E-02	9.80E-02	0.7508053
Mean =	-0.3158683	5.99E+07	3.05E-03	3.54E-03	1 14E-02	3.22E-02	1.71E-02	2.44E-02	0.1869751
Std Deviation =	1.250229	4.78E+07	3.16E-03	3.67E-03	1.18E-02	3.34E-02	1.78E-02	2.53E-02	0.1937021
Variance =	1.563073	2.28E+15	9.96E-06	1.34E-05	1.40E-04	1.11E-03	3.15E-04	6.40E-04	3.75E-02
Skewness =	0.3027088	-0.3860756	0.7489147	0.7489147	0.7489147	0.7489147	0.7489147	0 7489147	0.7489147
5% Perc =	-2.210462	26.89178	1.29E-09	1.50E-09	4.85E-09	1.37E-08	7.27E-09	1.04E-08	7.93E-08
10% Perc =	-1.894945	328.6091	1.34E-08	1 55E-08	5 02E-08	1.41E-07	7 52E-08	1.07E-07	8.20E-07
15% Perc =	-1.646202	2475 628	1.10E-07	1.28E-07	4.14E-07	1.17E-06	6.22E-07	8.85E-07	6.77E-06
20% Perc =	-1.436049	15322.15	6.49E-07	7.54E-07	2.43E-06	6.86E-06	3.65E-06	5.20E-06	3.98E-05
25% Perc =	-1.251393	97078.3	4.46E-06	5.19E-06	1.68E-05	4.72E-05	2.51E-05	3.58E-05	2.74E-04
30% Perc =	-1.088553	671306	2.88E-05	3.37E-05	1.08E-04	3.04E-04	1.63E-04	2.33E-04	1.77E-03
35% Perc =	-0 9373378	4672775	1.91E-04	2 22E-04	7.17E-04	0.0020186	1.08E-03	1.53E-03	1.17E-02
40% Perc =	-0.7779503	3.61E+07	9 11E-04	1.06E-03	3.42E-03	9.63E-03	5.12E-03	7.30E-03	5.59E-02
45% Perc =	-0 61658	1.00E+08	1.71E-03	1.99E-03	6.43E-03	1.81E-02	9 63E-03	1.37E-02	0.1050709
50% Perc =	-0.4464388	1 00E+08	2.37E-03	2.76E-03	8.91E-03	2.51E-02	1.34E-02	1.90E-02	0.1457148
55% Perc =	-0.2678512	1.00E+08	2.92E-03	3.40E-03	1.10E-02	3 09E-02	1.64E-02	2.34E-02	0.1793843
60% Perc =	-8.16E-02	1.00E+08	3.46E-03	4.03E-03	1.30E-02	3.66E-02	1.95E-02	2.78E-02	0.2126279
65% Perc =	0.1202459	1.00E+08	4.04E-03	4.69E-03	1.52E-02	4.27E-02	0.0227105	3.23E-02	0.2476967
70% Perc =	0.3362877	1.00E+08	4.61E-03	5.35E-03	1.73E-02	4.87E-02	2.59E-02	3.69E-02	0.282773
75% Perc =	0.5670417	1.00E+08	5.29E-03	6.14E-03	1 98E-02	5.59E-02	2.97E-02	4.24E-02	0.324368
80% Perc =	0 8286361	1.00E+08	6.02E-03	7.00E-03	2.26E-02	0.0636519	3 39E-02	0.0482503	0.3695135
85% Perc =	1.127424	1.00E+08	6.85E-03	7.96E-03	2.57E-02	7.24E-02	0.0385269	5.49E-02	0.4202015
90% Perc =	1.476486	1.00E+08	7.86E-03	9.13E-03	2.95E-02	8.31E-02	4 42E-02	6.30E-02	0.4824037
95% Perc =	1.921461	1.00E+08	9.17E-03	1.07E-02	3.44E-02	9.69E-02	5.16E-02	7.35E-02	0.5626578

Table G6 - Mascarpone risk assessment, shelf life storage at 10 °C

	Susceptible population								Susceptible population
Name	Cancer	Transplant	AIDS	Diabetes	Pregant	Kidney	< 30 days	> 60 years	Total
Minimum =	2.31E-12	7.30E-12	5 77E-12	1 20E-11	1.55E-11	4.47E-13	1.69E-12	9.94E-12	4.33E-11
Maximum =	8.89E-02	0.2805187	0.2217851	0.4614532	0.5943489	1.72E-02	6.49E-02	0.3822237	1.664177
Mean =	2.21E-02	6.99E-02	5.52E-02	0.114917	0.1480124	4.28E-03	1.62E-02	9.52E-02	0.4144346
Std Deviation =	2.29E-02	7.24E-02	5.72E-02	0.1190515	0.1533376	4.43E-03	1.67E-02	9.86E-02	0.4293452
Variance =	5.26E-04	5.24E-03	3.27E-03	1.42E-02	2.35E-02	1.96E-05	2.80E-04	9.72E-03	0.1843373
Skewness =	0.7489147	0.7489147	0.7489147	0.7489147	0.7489147	0.7489147	0.7489147	0.7489147	0.7489147
5% Perc =	9.39E-09	2.96E-08	2.34E-08	4.88E-08	6.28E-08	1.82E-09	6.85E-09	4.04E-08	1.76E-07
10% Perc =	9.71E-08	3.07E-07	2.42E-07	5.04E-07	6.50E-07	1.88E-08	7.09E-08	4.18E-07	1.82E-06
15% Perc =	8.02E-07	2.53E-06	2.00E-06	4.16E-06	5.37E-06	1.55E-07	5.86E-07	3.45E-06	1.50E-05
20% Perc =	4.71E-06	1.49E-05	1.18E-05	2.45E-05	3.15E-05	9.11E-07	3.44E-06	2.03E-05	8.82E-05
25% Perc =	3.24E-05	1.02E-04	8.09E-05	1.68E-04	2.17E-04	6.27E-06	2.37E-05	1.39E-04	6.07E-04
30% Perc =	2.09E-04	6.65E-04	5 26E-04	1.09E-03	1.41E-03	4.08E-05	1.54E-04	9.07E-04	3.95E-03
35% Perc =	1.39E-03	4.39E-03	3.47E-03	7 20E-03	9.29E-03	2.69E-04	1.01E-03	5.98E-03	2.60E-02
40% Perc =	6.62E-03	2.09E-02	1.65E-02	3.43E-02	4.42E-02	1.28E-03	4.83E-03	0.0284478	0.1238599
45% Perc =	1.24E-02	3.93E-02	3.10E-02	6.46E-02	8.32E-02	2 40E-03	9.08E-03	5.35E-02	0.232892
50% Perc =	1.73E-02	5.44E-02	4.30E-02	8.96E-02	0.1153501	3.33E-03	1.26E-02	7.42E-02	0.3229802
55% Perc =	2.12E-02	6 70E-02	5.30E-02	0.1102516	0.1420034	4.11E-03	1.55E-02	9.13E-02	0.3976094
60% Perc =	2 52E-02	7.95E-02	6 29E-02	0.1306835	0.1684542	4.87E-03	1.84E-02	0.1083323	0.4716718
65% Perc =	2.93E-02	9.25E-02	7.32E-02	0.1522371	0 1960805	5.67E-03	2.14E-02	0.1260987	0.5490254
70% Perc =	3.35E-02	0.1056755	8.35E-02	0.1737954	0.2239	6.47E-03	2.44E-02	0.1439893	0.62692
75% Perc =	3.84E-02	0.1211916	0.0958171	0.1993602	0.2567747	7.42E-03	2.80E-02	0.1651309	0.718969
80% Perc =	4.37E-02	0.138059	0.1091529	0.2271071	0.2925126	8.46E-03	3.19E-02	0.1881138	0.8190352
85% Perc =	0.0497485	0.1569972	0.124126	0.2582605	0.3326379	9.62E-03	3.63E-02	0 2139183	0.9313862
90% Perc =	5.71E-02	0.1802375	0.1425003	0.2964907	0.3818782	1.10E-02	4.17E-02	0.2455845	1.069259
95% Perc =	0.0666142	0.2102223	0.166207	0.3458157	0.4454086	1.29E-02	4.86E-02	0.2864407	1.247144

Table G7 - Mascarpone risk assessment, 21 day shelf life

Name	Level at end of storage	Level at time of consumption	Number of serves/annum	Probability of infection (per meal)	Dose	Susceptible population Total	General population Total
Minimum =	-2.977539	1 49E-03	91155.38	0	3.88E-02	0	0
Maximum =	3 050873	1.00E+08	2096558	1.13E-04	6.05E+09	0.126469	0.0220787
Mean =	-0.3169383	46872.02	286597 3	5.40E-08	2888491	5.70E-05	9.95E-06
Std Deviation =	1 249077	1253454	196938.6	1.60E-06	8.55E+07	1.53E-03	2.68E-04
Variance =	1 560195	1.57E+12	3.88E+10	2.56E-12	7.32E+15	2.35E-06	7.16E-08
Skewness =	0.3026393	58.75808	2.671605	53.09044	53.09139	62.54059	62.54059
5% Perc =	-2.209924	0.2381908	111898	1.94E-13	10.38207	2.32E-10	4.05E-11
10% Perc =	-1.892129	0.7087194	124040.5	5.67E-13	30.31429	6.66E-10	1.16E-10
15% Perc =	-1.643641	1.501058	135285.9	1.23E-12	65.96319	1.45E-09	2 53E-10
20% Perc =	-1.434505	2 6024	146481.8	2.16E-12	115 5291	2.55E-09	4.45E-10
25% Perc =	-1.251689	4.143193	158020.1	3.46E-12	185.2388	4.22E-09	7.37E-10
30% Perc =	-1.088363	6.322362	170129.9	5.41E-12	288.7915	6.66E-09	1.16E-09
35% Perc =	-0 9358481	9.459143	183015.2	8.26E-12	441.5464	1.02E-08	1.77E-09
40% Perc =	-0 7795467	14.39809	196888.5	1.27E-11	676.514	1.57E-08	2.75E-09
45% Perc =	-0.6132758	21.49173	212018.7	1.91E-11	1020.511	2.37E-08	4.13E-09
50% Perc =	-0.4448341	32.19248	228614	2.90E-11	1550.804	3.53E-08	6.16E-09
55% Perc =	-0.2664284	48.33975	247009	4.40E-11	2355.123	5.36E-08	9.36E-09
60% Perc =	-7 95E-02	75.53806	267562.1	6.79E-11	3629.507	8 36E-08	1.46E-08
65% Perc =	0.1193618	119.2552	290781	1.07E-10	5713.224	1.33E-07	2.33E-08
70% Perc =	0.3345355	198.8206	317240	1.78E-10	9529.255	2.20E-07	3.84E-08
75% Perc =	0.5654476	348.7723	347841.3	3.21E-10	17152 84	3.86E-07	6.75E-08
80% Perc =	0.8262392	698.9882	383652 3	6.37E-10	34064.59	7.32E-07	1.28E-07
85% Perc =	1.116611	1566.223	431702 7	1.44E-09	76929.61	1.69E-06	2.94E-07
90% Perc =	1.465945	4420 805	506971.3	3.83E-09	204676.6	4.88E-06	8.52E-07
95% Perc =	1.923769	20617.56	655909.7	1.87E-08	998205.3	2 24E-05	3 92E-06

Table G8 - Mascarpone detailed risk assessment correlations

Dose / Probability of infection (per meal)

Rank	Name	Correlation Coefficient
1	Time of consumption	0 8304123
2	Temperature	0 2017931
3	Lag phase	-0.3538089
4	Serving size	0.1245999
5	Production pH	1.88E-02
6	Time of contamination	-4.83E-02
7	Consumer salt concentration	8.19E-03
8	Contamination level	0.1058288
9	Production %NaCl	7.95E-03
10	Frequency of contamination	-1.34E-02
11	Distribution temperature	1.42E-02
12	Distribution pH	1.71E-02
13	Distribution salt concentration	3.53E-03
14	Consumer temperature	-4.19E-03
15	Consumer pH	-8.08E-03

Number of listeriosis cases

Rank	Name	Correlation Coefficient
1	Time of consumption	0.8290305
2	Production Temperature	0.1980824
3	Frequency of contamination	0.1144512
4	Lag phase	-0.3543057
5	Time of contamination	-4.98E-02
6	Production pH	0.0167845
7	Serving size	1.17E-02
8	Consumer pH	-3.55E-03
9	Contamination level	0.1051936
10	Production %NaCl	0.0105101
11	Distribution temperature	1.27E-02
12	Distribution pH	1 52E-02
13	Distribution salt concentration	3.73E-03
14	Consumer temperature	-3.61E-03
15	Consumer salt concentration	9.39E-03

Level at end of storage

Rank	Name	Correlation Coefficient
1	Contamination level	0.997763
2	Time of contamination	-7.63E-03
3	Lag phase	-7.71E-03
4	Production Temperature	-1 84E-02
5	Production pH	-1.64E-03
6	Production %NaCl	8.43E-03
7	Distribution temperature	-5 88E-03
8	Distribution pH	1.45E-03
9	Distribution salt concentration	-9.82E-03

Level at time of consumption

Rank	Name	Correlation Coefficient
1	GT= / Time of consumption	0.837478
2	Production Temperature	0.2017783
3	Lag phase	-0.3560509
4	Production pH	1.89E-02
5	Time of contamination	-4.85E-02
6	Serving size	9.84E-03
7	Contamination level	0.1078987
8	Production %NaCl	1.01E-02
9	Frequency of contamination	-1.46E-02
10	Distribution temperature	1.30E-02
11	Distribution pH	1.61E-02
12	Distribution salt concentration	2.03E-03
13	Consumer temperature	-2.03E-03
14	Consumer pH	-8.05E-03
15	Consumer salt concentration	8.49E-03

Appendix H – Profiling of Mascarpone contaminant

Identification

During one of the routine sampling periods of Mascarpone cheese, a large number (1.8×10^7 cfu/g) of colonies were detected on EMB agar. These colonies appeared like typical *E. coli* colonies, with distinctive green metallic sheen from reflected light and purple centres from transmitted light. A second sample was obtained from the same batch with the same 'Use-by' date on it, and 6.5×10^7 cfu/g of the same *E. coli*- like colonies on EMB agar were detected. Further identification using an API 20E strip (a commercial kit based on 20 biochemical tests – Appendix A) showed a 60% probability of the isolate being *Hafnia alvei* (Table H.1). It was identified through 2 different substrate utilisation profiles, one a 46% correlation, the other 14%. The 'Tests against' indicates the substrate utilisation tests which normally return an alternate result for the identified organism. The testing also showed there was a low probability the organism was *E. coli* (4%). *Hafnia* strains do not normally ferment lactose and therefore would not be expected to give the *E. coli*-type colonies on EMB, however plasmid-mediated lactose positive strains may occur (Albert *et al.*, 1992).

Table H.1 – Biochemical tests for identification of Mascarpone cheese contaminant

Identification	Correlation (%)	Tests against	
Hafnia alvei	46	2	AMY (5%), ARA (18%)
Hafnia alvei	14	1	RHA (97%)
Escherichia fergusonii	35	2	IND (99%), RHA (87%)
Escherichia coli	4	2	IND (77%), AMY (12%)

AMY = amylase, ARA = arabinose, RHA = rhamnose, IND = indole

Growth modelling

An experiment was conducted to profile the growth rate response to temperature of the *Hafnia* isolate found on the Mascarpone cheese during routine sampling. The organism was isolated on EMB agar, transferred to TSB and grown overnight at 25°C. Stock cultures were maintained as outlined in Appendix C1, and the culture for growth experiments conducted according to the procedure outlined in Appendix C2. Growth rate experiments were conducted with a Temperature Gradient

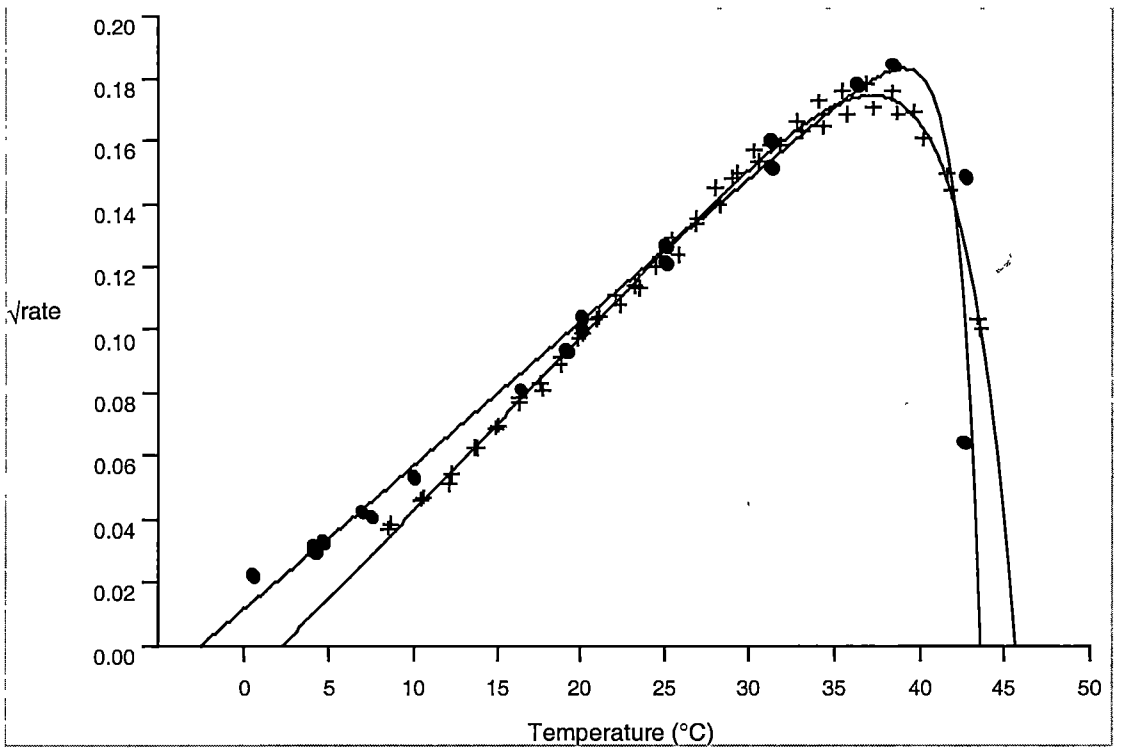
Incubator (TGI - Appendix A). With this equipment, growth rates could be measured simultaneously over 30 different temperature conditions. Fifteen mL aliquots of sterile TSB were aseptically dispensed into L-tubes (Appendix A), the tubes placed into the TGI and allowed to equilibrate to temperature overnight. The TGI was placed into a constant temperature room (20 ± 0.5°C), to minimise temperature fluctuations during the experiment.

Bacterial growth was assessed by the measurement of the change in transmittance (Δ%T), determined at 540 nm using a Spectronic 20 spectrophotometer (Appendix A). Full scale range (100%T) was set with a blank of uninoculated TSB, and sufficient inoculum was added to each L-tube so as to bring the initial %T reading to between 80-90 %T. Measurement times were chosen to correspond with changes of 5 - 10 %T and continued until %T fell to ~5%. Temperatures of the broth within the L-tubes was determined at the conclusion of the experiment, with the mean value from 5 readings used. Duplicate growth experiments were conducted, with the mean value used for the generation of fitted values.

The time *vs* %T data was entered into a SAS PROC non-linear regression routine (Appendix A2), generating the Gompertz parameters necessary for calculation of the generation time according to Eqn A3. (Appendix D). A square plot (Section 1.4.2.2) was constructed, and a curve of best fit obtained by non-linear regression (Ultrafit – Appendix A1), using the Ratkowsky *et al.* (1983) four parameter square root model to calculate T_{min} (and T_{max}) values. These are the theoretical temperature below (and above) which no growth can occur, usually 1 – 2 °C below (and above) the observed minimum (and maximum) temperature for growth.

Growth rate profiling of the Mascarpone contaminant showed the T_{min} value to be 2.2 ± 0.5°C, with growth observed down to 8°C (Fig 6.8). The T_{max} value was estimated to be 45.6 ± 0.2°C. The generation time at 10°C was calculated to be in the order of 8 hr, therefore showing the isolate to be psychrotrophic. The growth data generated in this study was compared with data measured for *Enterobacter hafniae* (since reclassified as *Hafnia alvei*) from Langeveld & Cuperus (1980). Fitting of this data showed a T_{min} value of -2.5°C with growth observed down to 0.5°C (Fig H.1).

Figure H.1 - Comparison of Mascarpone isolate (+) and comparison with growth data from Langeveld & Cuperus (1980) for *Enterobacter hafniae* (•)



These growth profiles were generated under optimal conditions of pH and a_w in laboratory media. Therefore, the lower pH of the Mascarpone cheese curd may be a limiting factor for growth within the cheese itself, and the growth rate may be slightly less. However, given the ability of the organism to grow at low temperatures, it would be expected that it would be able to grow during the shelf life of the Mascarpone.

Hafnia alvei as a potential pathogen

H. alvei appears to be part of the normal human enteric biota, since it is frequently isolated from abdominal specimens, but published reports indicate it may also be an opportunistic pathogen. There have been a significant number of gastroenteritis cases attributed to this organism (Westblom & Milligan, 1992; Ridell *et al.*, 1994), particularly in children (Reina *et al.*, 1993). It has also been shown that *H. alvei* shares some of the same virulence factors with enteropathogenic *E. coli* (Albert *et al.*, 1992). These results suggest that there is the potential for this organism to cause diarrhoeagenic disease (Albert *et al.*, 1991).

H. alvei has been found in raw ewe's milk (Gaya *et al*, 1987) and hard cheese made from goat's milk (Tornadijo *et al.*, 1993). Factory 'B' also manufactured some Goats milk products, therefore the lack of physical separation between the receival and processing areas may have contributed to this contamination event. The organism also occurs in animals and birds, and also in natural environments such as soil, sewage, water and dairy products. This indicates that factory hygiene must also incorporate pest control and water treatment elements.

Irrespective of the contamination source, the number of *H. alvei* detected in the final product was at a level in the Mascarpone cheese that suggested some uncontrolled factor during production or temperature abuse allowed growth of the organism to occur. Tornadijo *et al* (1993) found that numbers of *H. alvei* increased in the curd and in 1 week old cheese, where it contributed up to 75% of the isolates recovered from goats cheese. They found that *H. alvei* gained access to raw milk as a result of lack of hygiene during milking and its manipulation (Tornadijo *et al*, 1993).